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Ultrastructural and Biochemical Studies on the Epiphytic Subaerial Green Alga *Phycopeltis* *Epiphyton* Millardet.

Barry Harman Good

Louisiana State University and Agricultural & Mechanical College

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ULTRASTRUCTURAL AND BIOCHEMICAL STUDIES ON
THE EPIPHYTIC SUBAERIAL GREEN ALGA
PHYCOPELTIS EPIPHYTON MILLARDET.

THE LOUISIANA STATE UNIVERSITY AND
AGRICULTURAL AND MECHANICAL COL., PH.D., 1978

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Ultrastructural and Biochemical Studies
on the Epiphytic Subaerial Green Alga
Phycopeltis epiphyton Millardet

A Dissertation

Submitted to the Graduate School of the
Louisiana State University
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Botany

by
Barry Harman Good
B.S., Northeast Louisiana State College, 1970
M.S., Northeast Louisiana University, 1972
August, 1978

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Abstract

Morphology of vegetative cells, reproductive structures and reproductive motile cells of Phycopeltis epiphyton Millardet (Chroolepidaceae, Chlorophyta) was studied by light microscopy, scanning and transmission electron microscopy. Cell wall components were studied by chemical analyses.

The monostromatic pseudoparenchymatous algal thallus is foliicolous and supracuticular. Subtending host tissues exhibit no anatomical response to presence of the alga. Senescing filaments of older thalli occasionally produce new thalli. Each vegetative algal cell has a nucleus with a single nucleolus and contains numerous parietal chloroplasts which produce starch grains and plastoglobuli. Thylakoid membranes do not form discrete higher plant-type grana; pyrenoids are absent. Elongate mitochondria are frequently associated with other cellular organelles. Cytoplasmic inclusions containing the lipid pigment haematochrome are abundant and not membrane bound. Cell branching is initiated by a terminal cell wall infold. Septal formation probably occurs by a combination of infurrowing and cell plate formation. A central pit area which contains a ring of lightly stained material and simple plasmodesmata is present within each septum. A densely staining material present within the inner and outer zones of cell walls resists acetolysis, is degraded by chromic acid, is unaffected by ethanolamine and exhibits secondary fluorescence when stained with the fluorochrome

Primuline. These characteristics and infrared absorption spectra indicate that the material is a sporopollenin and that it is a major cell wall component. Tests for cellulose, chitin, and lignin are negative, and little if any silica is present. Negative results in tests for cellulose may be due to a masking effect by sporopollenin. Comparison of fine structure of cell walls of P. epiphyton, pollen grains and algal cells (known to contain sporopollenin) supports the suggestion that sporopollenin deposition on "unit membranes" is universal. Morphological similarity among sporopollenin lamellae in P. epiphyton, pollen grains, spores of land plants, and trilaminar sheaths in Chlorella, Coelastrum, Pediastrum, and Scenedesmus indicates that the structures may be analogous. As in pollen grains, sporopollenin in P. epiphyton may provide protection against desiccation and parasitism, and be involved in adhesion to host plants and in adhesion between adjacent thallus filaments.

Zoosporangium ontogeny occurs apically in pedicel cells. A septal ridge demarcates the pedicel from its rugulose apical end and indicates at least partial development of the plasmodesmata-containing zoosporangium-pedicel septum. As the zoosporangium enlarges, a protuberance (future escape pore) forms terminally, subterminally, or laterally. Early in zoosporangial abscission bulging septal regions of zoosporangium and pedicel initiate lateral wall rupturing, followed by partial splitting of the zoosporangium-pedicel septum. A centrally located hatch-like protuberance emerges from zoosporangium and pedicel, and together with a slight septal bulging is involved in final separation of the zoosporangium.

In quadriflagellate zoospores, apically inserted keeled flagella terminate in two upper and two lower parallel, overlapping basal bodies. Upper basal bodies insert into the apical papilla from opposite directions, as do the lower basal bodies. Two multilayered structures are present, with one multilayered structure being associated with each upper basal body. Eye-spots are absent.

Gametes are biflagellate. Flagella are keeled and insert into an apical papilla from opposite directions. A distal band is above the basal bodies. Two multilayered structures are present, one multilayered structure being associated with each basal body. Eye-spots are absent.

Based on the fine structure of motile cells and fossil evidence, P. epiphyton is probably not on a direct evolutionary line to archegoniate plants.

General Introduction

This dissertation is primarily a study on the ultrastructure of Phycopeltis epiphyton and may be conveniently organized into three sections. Chapter I will introduce the alga including its general morphology and vegetative ultrastructure. Unexpected and intriguing data on the alga's cell wall were obtained during the course of the investigations reported in Chapter I and these data are presented in Chapter II. Chapter III includes observations on the ultrastructure of reproductive structures and the motile cells produced within them. Also, the possible phyletic significance of the observations will be discussed in relation to the Chroolepidaceae, other green algae, and archegoniate land plants.

Although each chapter will include a brief specific introduction, this general introduction has been prepared to familiarize the reader with an organism which is virtually unknown, even to most phycologists, and which is an intriguing species in a small and unusual family of green algae. For such an alga, a general introduction to the characteristics of the genus and the family is required to provide a basic understanding of the alga, related genera, and the overall position of the family in the Chlorophyta. In this general introduction, an attempt will be made to present a clear picture of the research organism, although, as will be discussed, the generalized life cycle of Phycopeltis has never been reported and the phylogenetic position of the entire family is a

topic under discussion in current phycological literature. Further, although all of the taxa in the family stimulate immediate interest because of their subaerial habitats, the total amount of published experimental research on this algal group is not great.

The Family

An introduction to the Chroolepidaceae begins with two basic questions. First, what is the correct name for the family and, second, which genera belong in the family? Despite the fact that "Trentepohliaceae" occurs more frequently in the literature, Silva who traced the numerous and confusing circumscriptions that have occurred within this algal group (1950) believes (personal correspondence with Dr. R. L. Chapman) that Chroolepidaceae is the earliest legitimate family name for these algae. The historical precedence of "Chroolepidaceae" has also been cited by Papenfuss (1962). The second question, that of which genera are in the family, is perhaps more difficult to answer because of the varied opinions among phycologists. Printz (1939, 1964) and Bourrelly (1966) limit the Chroolepidaceae to only subaerial algae, whereas other authors (e.g., Smith, 1950; Fritsch, 1965; Prescott, 1968) include certain aquatic taxa. It should be noted that Printz has published the most comprehensive work on the Chroolepidaceae, although Dr. Rufus Thompson, Department of Botany, University of Kansas, has also completed detailed, as yet unpublished, studies of these algae. The restriction of the family to subaerial genera is reasonable because these subaerial algae are also similar in having the following: 1. cross-walls containing centrally located plasmodesmata which form a "pit"; 2. haematochrome; 3. asexual

reproductive structures which are highly differentiated as compared to vegetative cells; 4. zoosporangia which detach via a double ring septum and are dispersed by wind and rain; and 5. motile reproductive cells which have 2 or 4 keeled flagella (Printz, 1939; Bourrelly, 1966; Graham and McBride, 1975; Chapman and Good, 1977, 1978; Chappell et al., 1978). The inclusion of aquatic taxa in the Chroolepidaceae based on the presence of more or less differentiated reproductive structures is unjustified because, with the exception of the presence of haematochrome (a pigment which occurs in many green algal families), none of the other main characteristics has been observed in the aquatic forms (Smith, 1950; Fritsch, 1965; Chappell et al., 1978).

The Genera

As mentioned earlier, Printz (1939) restricted the Chroolepidaceae to subaerial taxa, including Cephaleuros, Phycopeltis, Physolinum, Stomatochroon, and Trentepohlia; however, Flint (1959) eliminated the generic name Physolinum by returning P. monile to the genus Trentepohlia as T. monile. A few words about these four sub-aerial genera will further introduce the family and facilitate comparison among its members.

Cephaleuros occurs in tropical and subtropical regions of the world and parasitizes the leaves, stems, or fruits of hundreds of species of vascular plants. The alga usually grows beneath the cuticle or epidermis of its plant host, forming a disc-shaped vegetative thallus two or more cell layers thick, composed of anastomosing filaments. The thallus produces rhizoidal ventral filaments,

sterile dorsal trichomes, and reproductive (i.e. sporangia-bearing) branches. The trichomes and reproductive branches emerge through the cuticle of the plant host and the alga causes a wound response in subtending tissues.

Phycopeltis occurs abundantly in tropical and subtropical regions, and has been found in a few temperate regions as well (Printz, 1939; Suematu, 1957). Phycopeltis is epiphytic on the leaves of many vascular plants and its disc-shaped vegetative thallus, like that of Cephaleuros, is composed of anastomosing filaments. In contrast to Cephaleuros, Phycopeltis is always found on (never beneath) the cuticle of its host plant and its vegetative thallus, which is one cell layer thick, does not produce rhizoids or sterile hairs. Sporangia are borne singly on short unbranched reproductive filaments. In addition, the alga does not cause a wound response in subtending host tissues.

Stomatochroon is the most unusual and least reported of all the chroolepidaceous algae. The alga, a highly reduced form consisting of a few cells, inhabits the leaf stomatal apertures of many different tropical plants (Palm, 1934).

Trentepohlia, perhaps the best known member of this family, commonly grows on bark and rock in tropical, subtropical, and temperate world regions. Its vegetative thallus, composed of uniseriate and non-anastomosing branched filaments, does not have a disc shape and rhizoidal filaments are absent. Sporangia are borne terminally on single-celled reproductive filaments.

Although the Chroolepidaceae occur as free-living algae, it should be noted that Cephaleuros, Phycopeltis, and Trentepohlia are recorded to occur as phycobionts in 109 foliicolous lichen species, particularly in the Strigulaceae, Arthoniaceae, and Opegraphaceae (Santesson, 1952).

Species Identification

In addition to the question of which genera should be assigned to the Chroolepidaceae, the identification of species is also problematic. The only significant taxonomic reference on the different species of Phycopeltis is that of Printz (1939), but his descriptions and keys (containing 11 species) were of limited usefulness in attempts to identify the species of Phycopeltis that were collected in Louisiana. Because of this, herbarium specimens from the University of California (Berkeley) and the holotype of Phycopeltis hawaiiensis King from the Bernice P. Bishop Museum (Hawaii) were obtained for comparison with the Louisiana material. Not only was the herbarium material of little aid in identifying the Louisiana specimens, but it was subsequently determined that P. hawaiiensis was not a Phycopeltis species at all, but rather a Cephaleuros species (Chapman and Good, 1976).

As mentioned earlier, Dr. Rufus H. Thompson has completed detailed, as yet unpublished, studies of the chroolepidaceous algae. In view of Dr. Thompson's work, Phycopeltis specimens from Louisiana were sent to him for identification. He identified the Phycopeltis species used in this investigation as P. epiphyton Millardet and in addition made available his proposed key (unpublished) containing 17

descriptions of Phycopeltis species. The Louisiana specimens proved to be useful to Dr. Thompson in eliminating questionable taxa from his key (personal correspondence). Publication of Dr. Thompson's studies on chroolepidaceous algae will surely benefit other researchers working with these organisms.

The Life Cycle

In the preceding paragraphs, no reference was made to the generalized life cycle of the Chroolepidaceae. The entire subject, as reported in the literature, is contradictory. Although the Phycopeltis life cycle per se, has not been described, Printz (1939) described the generalized life cycle of the entire family. During this cycle, asexual reproduction can occur either by fragmentation of the thallus or more frequently by zoospores which are formed in stalked or hooked zoosporangia. Within each zoosporangium several zoospores are produced but are not released until the zoosporangium detaches from its stalk and is dispersed by wind or rain. The zoospores immediately give rise to a new vegetative thallus. Gametes are produced in gametangia that are slightly modified vegetative cells of the thallus. Syngamy occurs and the zygote immediately divides initiating a new vegetative thallus. In contrast to Printz's report, the life cycles of Cephaleuros and Stomatochroon were reported by Thompson (1959) to be as follows:

"Usually only 8 quadriflagellate zoospores are produced per sporangium. Sporangiochore initials often develop into akinetes which, upon germination,

may also produce quadriflagellate zoospores, 8 to 16 in number. Gametangia are sessile and dorsal on the vegetative filaments. They are variable in size and produce 8 to 32, usually 16, biflagellate isogametes. Syngamy may be extra- or intra-gametangial. In either case the zygote germinates immediately to produce a dwarf sporophyte plant. This consists of an attaching stalk cell bearing one or more suffultory cells each with a small dehiscent microsporangium. Four quadriflagellate microzoospores are produced per sporangium. All evidence points to the occurrence of meiosis in microzoospore formation."

Although not devoted to an investigation of the life cycle of Phycopeltis, this dissertation includes observations on the number of reproductive motile cell flagella. This information, which will be discussed in Chapter III, is consistent with the work of Thompson (1959), but not with that of Printz (1939).

Phylogenetic Position

In recent years, the use of biochemistry and ultrastructure for studying the phylogeny of green algae has resulted in major revisions of classical phylogenetic schemes. Although it is commonly believed that the green algae have phyletic affinities with archegoniate land plants, the question of which phyletic line within the green algae accomplished this transition is still controversial and

has been the subject of numerous investigations (e.g., Pickett-Heaps, 1969, 1972; Pickett-Heaps and Marchant, 1972; Frederick et al., 1973; Floyd and Salisbury, 1977). A complete discussion of this topic as well as a detailed review of the numerous papers published on the biochemistry and ultrastructure of green algae is beyond the scope of this general introduction. Despite the extensive amount of available information on the green algae in general, a clear phyletic placement of the Chroolepidaceae has not been possible (Stewart et al., 1973; Stewart and Mattox, 1975) because only a limited amount of ultrastructural and biochemical information has been reported for this family. Further, published ultrastructural observations on Phycopeltis are totally lacking. Because so little is known about the life cycle, biochemistry and ultrastructure of Phycopeltis, investigation of this alga has most certainly been warranted and the need for additional studies is clear.

Chapter I

General Morphology and Vegetative Ultrastructure

Introduction

Early workers published general morphological descriptions and line drawings of Phycopeltis species as well as other chroocolepidaceous algae (e.g. Agardh, 1824; Rabenhorst, 1868; Möbius, 1888; De Toni, 1889; Hariot, 1889; Karsten, 1891; Jennings, 1896; Oltmanns, 1905). In 1870 Millardet erected the species name Phycopeltis epiphyton for small yellow algal discs he found growing on the leaves of the gymnosperm Abies pectinata. Many of these early investigations were inadequate descriptions of these algae which resulted in much taxonomic confusion (e.g. Cunningham, 1879; Ward, 1884; Thomas, 1913).

This chapter will present the first description of the general ultrastructure of P. epiphyton, as well as confirm and illustrate with photomicrographs some of the earlier morphological descriptions and line drawings of Phycopeltis species. Cell division, septum formation, and filament branching will be discussed. In addition, the structure of cell walls in general and the central pit areas present in the septa in particular will be described, and the central pit areas will be compared to those found in the septa of Cephaleuros and Trentepohlia.

Materials and Methods

Phycopeltis epiphyton Millardet foliicolous on Sabal minor (Jacquin) Persoon and Osmanthus fragrans Loureiro was collected in March 1974 from Burden Research Plantation, Louisiana State University, Baton Rouge and in June 1975, from Locust Grove Cemetery, Bains, Louisiana. Specimens were examined or prepared (see below) within four hours of collection.

Light Microscopy

Small portions of fresh S. minor and O. fragrans bearing P. epiphyton were excised, fixed in FAA (1:1:18, vol/vol/vol) or Nawaschin (Craf) III for 24 hr and washed with water (Sass, 1958). The material was then softened in HF:H₂O (1:1, vol/vol) for approximately 110 hr, washed in running tap water 12 hr, dehydrated through a tertiary butyl alcohol series ending in xylene (100%), and embedded in paraffin. Sections (ca. 8 μ m thick) were produced with a Spencer 820 rotary microtome (American Optical Corporation) and stained either with safranin-fast green, iron-alum hematoxylin or tannic acid (Johansen, 1940; Sass, 1958). Whole mounts of living material were prepared by gently removing algal thalli from the upper surfaces of leaves with a small thin knife and placing them in a drop of distilled water. Sectioned and living specimens were observed and photographed with a Leitz Orthoplan microscope and Orthomat camera using Kodak, Panatomic-X film.

Transmission Electron Microscopy

Leaves of both S. minor and O. fragrans bearing P. epiphyton or individual algal thalli (see above) were successfully prepared by two different fixation and dehydration procedures. In the first method material was fixed for 2 hr in 4% glutaraldehyde in 0.2 M potassium phosphate buffer (pH 6.8) at 4C, rinsed 3X with buffer, postfixed for 2 hr in 1% osmium tetroxide in the same buffer at 4C, rinsed 3X, and dehydrated through a graded ethanol series (10-60% at 4C, 75-100% at 23C). In the second method (Salema and Brandao, 1973), material was fixed with 5% glutaraldehyde in 0.08 M Pipes buffer (pH 8.0) for 1 hr at 23C, rinsed 3X with 0.2 M Pipes buffer (pH 6.8) for 1 hr, postfixed with 2% osmium tetroxide in 0.18 M Pipes buffer (pH 6.8) for 2 hr at 23C, and rinsed 3X for 1 hr. The material was dehydrated through a graded acetone series (10-100%) at 23C. The fixed and dehydrated material was embedded in a "firm" mixture of Spurr's plastic (Spurr, 1969) or transferred to 100% propylene oxide and embedded in Epon 812. A third fixation procedure employed 5% (aqueous) potassium permanganate (KMnO_4) or 5% KMnO_4 in 0.2 M potassium phosphate buffer (pH 6.8) on ice for 1-2 hr. This fixed material was dehydrated through a graded ethanol series and embedded in Spurr's plastic. Thin sections (approximately 60-100 nm) were cut with a Dupont diamond knife using a Porter-Blum MT-II ultramicrotome, collected on naked copper grids or parlodion coated copper grids pretreated with 1% polybutene, stained with uranyl acetate (1-2% in acidified ethanol) and lead citrate (Reynolds, 1963), observed with an Hitachi HU-11A electron microscope (75kv), and photographed with Kodak 4489 Electron Microscope film.

Scanning Electron Microscopy

Small portions of leaves bearing P. epiphyton were excised, fixed with 4% glutaraldehyde in 0.02-0.12 M potassium phosphate buffer (pH 6.8) for 2 hr at 23C. The specimens were rinsed with buffer and dehydrated either through a graded ethanol-amy1 acetate series at 23C or through rapid chemical dehydration using acidified 2,2 dimethoxypropane (Muller and Jacks, 1975). Material dehydrated by the latter method was transferred to 100% acetone for 1 hr and then into amy1 acetate. All fixed and dehydrated specimens were dried with CO₂ in a Denton DCP-1 critical point dryer, mounted on aluminum stubs either with silver conducting paint or double-stick tape, coated in vacuo with a thin layer of gold (ca. 20-30 μ m) or gold-palladium, observed in JEOL JSM-2 or Hitachi S-500 scanning electron microscopes (15-25 kv), and photographed with Kodak Tri X Ortho film.

Observations

Phycopeltis epiphyton vegetative thalli on leaves of Sabal minor and Osmanthus fragrans were disc-shaped and less than 1 mm in diameter (Fig. 1-3). On each leaf, algal thalli of different sizes (and presumably different ages) were intermixed. In shady habitats the algal thalli were grass-green in color while in exposed habitats they were orange-red. The monostromatic thalli were adaxial and supracuticular on their leaf hosts and did not cause any apparent wounding reaction in subtending host tissues (Fig. 4). Each uniformly thick pseudoparenchymatous thallus was composed of laterally appressed filaments which bifurcated centrifugally (Fig. 5 and 6, 9). At times the alga would cover extensive areas of the leaf's surface; however, the thalli always abutted rather than overlapped (Fig. 7). Young marginal cells of algal thalli (both fresh, Fig. 3 and FAA-fixed, Fig. 7) were lighter in color than older more centrally located cells. Frequently, the older portions of filaments (those closest to the center of the thallus) appeared to be senescent. Occasionally, new thalli were initiated from these older portions of filaments (Fig. 8 and 10).

As seen in thin-section a typical vegetative cell contained an abundance of the non-membrane bound cytoplasmic pigment haematochrome (β carotene or derivative), as well as eukaryotic cellular organelles such as a single nucleus, a few parietal chloroplasts, plastoglobuli, a plasmalemma, and numerous ribosomes (Fig. 11). Not shown in

Figure 11 but also present in typical vegetative cells were dictyosomes, endoplasmic reticulum, a few elongate mitochondria, and small vacuoles. Some of these organelles are shown in subsequent figures. The outer parts of terminal, cross and lateral cell walls contained a densely staining material that will be discussed in Chapter II. Each vegetative cell contained one nucleus in which a single nucleolus was characteristically present (Fig. 12). In addition, it was frequently observed that mitochondria were closely associated with the nucleus (Fig. 13). Chloroplasts contained thylakoid membranes (which did not form discrete grana), plastoglobuli, and starch grains (Fig. 14). As compared to the chloroplasts in cells which appeared healthy, the number and volume of plastoglobuli increased in the chloroplasts of senescing cells. Pyrenoids were never observed.

Several stages in the ontogeny of naturally occurring (i.e. foliicolous) vegetative thalli from unicellular zoospores revealed the onset of cell "infolding" (the terms "infolding," "infolded," and "infold," are descriptive and, as will be discussed, do not connote the process involved). Immature thalli began as single cells (Fig. 15) which became involute as they enlarged. As the "infolds" of the cell wall progressively lengthened centripetally, the wide sinus that was initially present between adjacent walls of the "infold" (asterisks Fig. 15) became narrower (Fig. 16). Eventually a septum formed between cell wall "infolds" (Fig. 17). (The possible modes of septal formation will be discussed later.) As the thallus became larger, the basic pattern of cell wall "infolding" and subsequent septum formation was repeated until a pseudoparenchymatous thallus (less than 1 mm in diameter) composed of predominantly bifurcated filaments was formed

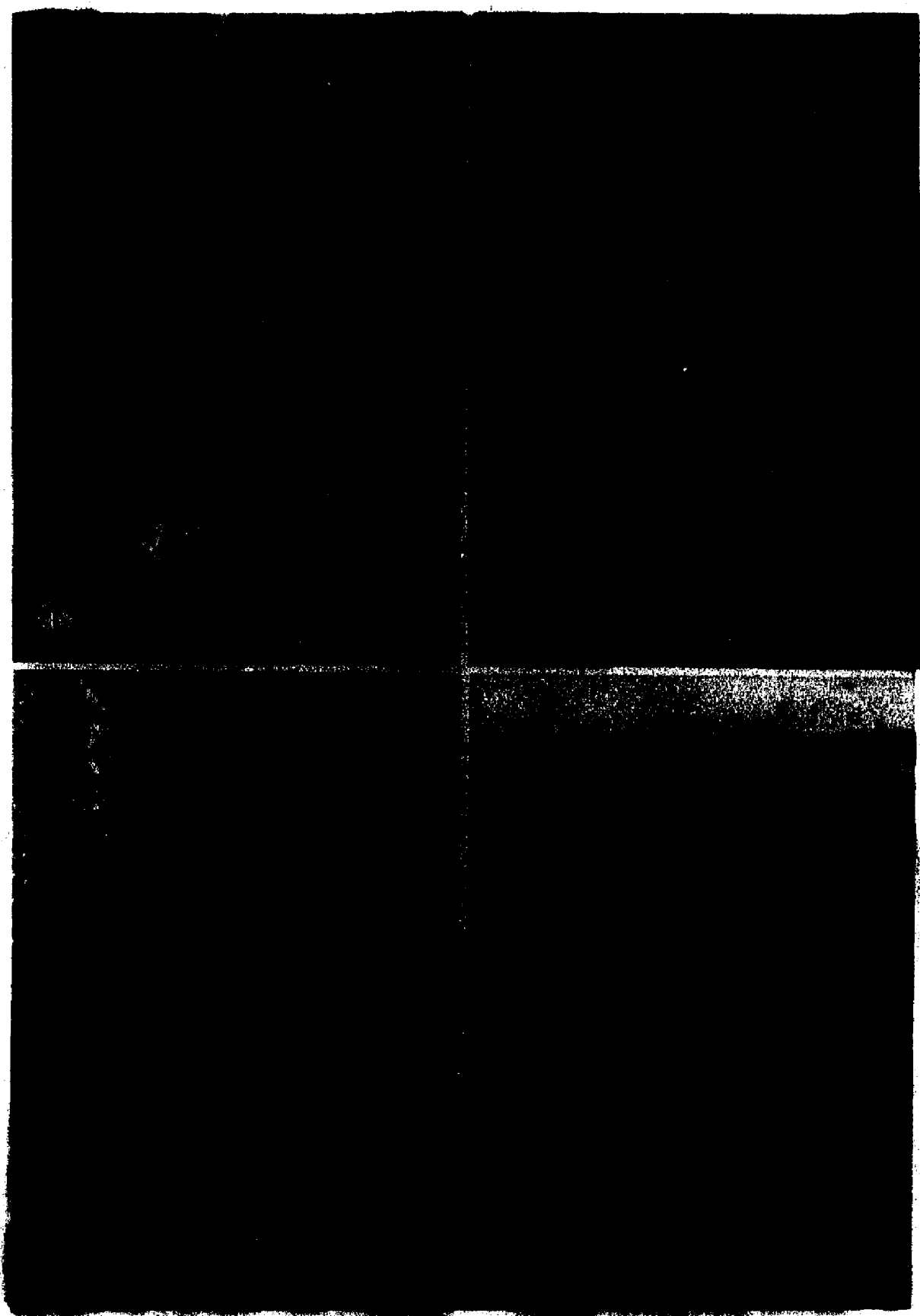
(Fig. 18). Meristematic marginal cells of a nearly mature thallus (Fig. 19-20) had "infolded" cell walls that represented future sites of septal formation. A slight plasmolysis during fixation, created a space ("S" in Fig. 20) between the cytoplasm and cell wall (however, cf. the vacuole in Fig. 23). The cell wall at the tip of "infolds" (e.g. Fig. 20) occasionally appeared to be thicker than the marginal cell walls.

Three slight variations on this basic branching pattern (see above) were observed in the region of the thallus between the apical margin and center (Fig. 21-24). In one type (Fig. 21), an "infold" of the cell wall was followed by formation of a septum which connected one lateral wall (LW) of the cell and the tip of the centripetally directed "infold" (asterisk). A second septum (between arrowheads) connected the other lateral wall with a region slightly above the terminal wall "infold." In another type of branching pattern (Fig. 22), septa connected opposite lateral walls at positions that were both slightly distal to the tip of the wall "infold." Further, a vacuole (V) was observed immediately beneath the tip of the "infold" (Fig. 23). Cell trifurcation (Fig. 24) was less frequently observed, but represented a third branching pattern. Apparently two cell wall "infolds" produced this trichotomy. (Asterisks denote the tips of the "infolds.")

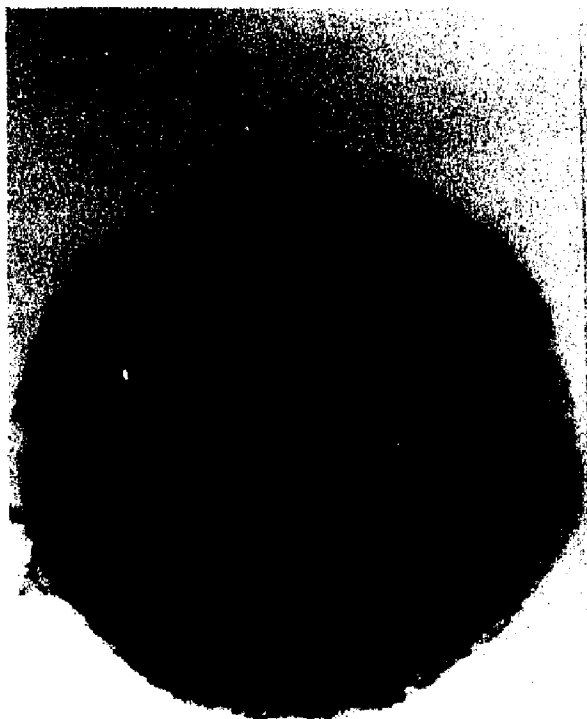
The septa characteristically had centrally located pit areas which contained plasmodesmata (Fig. 25-29). Each such pit area was distinct due to the presence of a bordering ring of lightly stained material (arrows), and to the relative thinness of the wall within the pit area as compared to the outer area of the septum (Fig. 25).

Although this differential thickening was a consistent feature, occasionally the septal wall within the pit was approximately the same thickness as that outside the border (Fig. 26). The plasmodesmata in each septum were always observed to be localized within the central pit area. These plasmalemma-lined plasmodesmata had a diameter of approximately 40-50 nm and were of the simple type; i.e. neither desmotubules nor other structures were ever observed (Fig. 27-29). The localization of the plasmodesmata in the central pit area was also evident in oblique sections (Fig. 27-29) wherein lightly stained margins surrounding the plasmodesmata were clearly visible (cf. Fig. 25). Densely stained plasmalemma produced the dark ring-like borders within the plasmodesmata (Fig. 27-29, cf. Fig. 25).

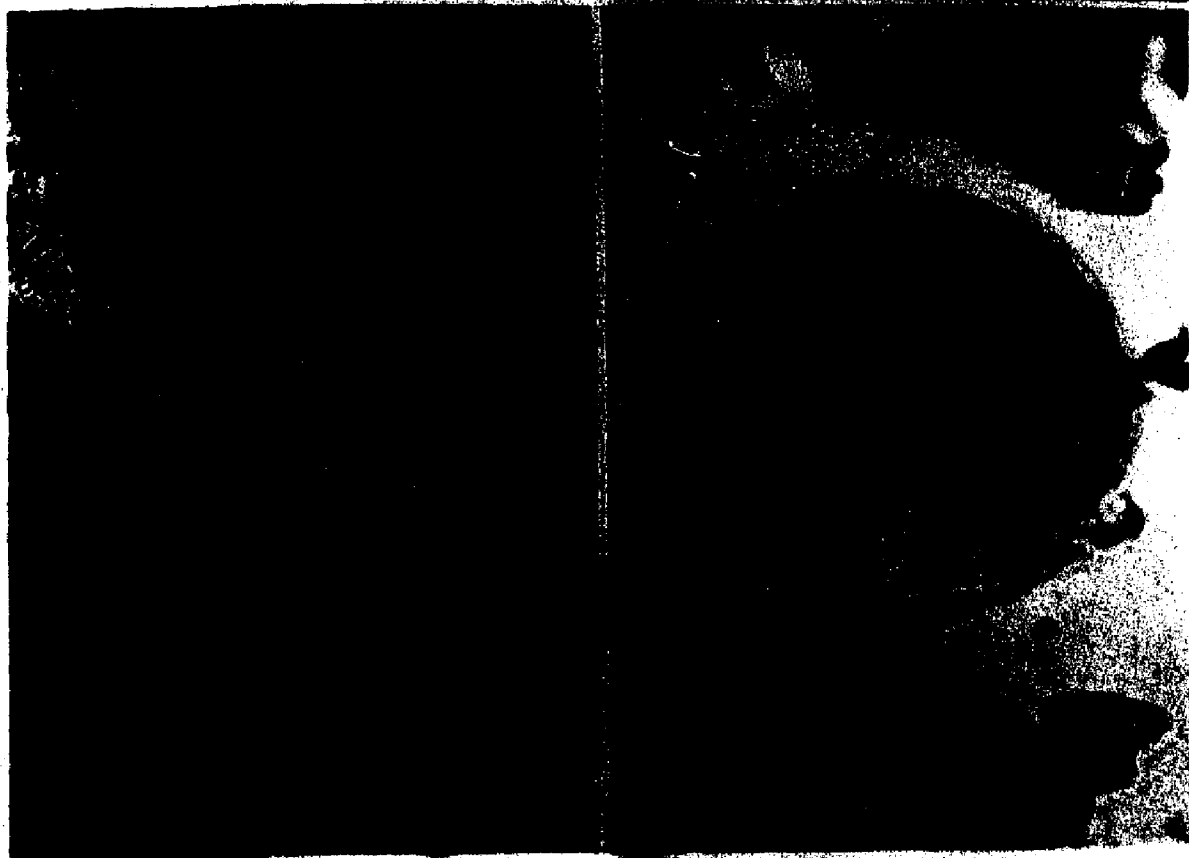
- Fig. 1. Numerous Phycopeltis epiphyton thalli (three marked with arrows) foliicolous on Sabal minor. X 7.
- Fig. 2. P. epiphyton thalli (arrows) foliicolous on Osmanthus fragrans. X 6.
- Fig. 3. Three P. epiphyton thalli foliicolous on S. minor. Note lighter marginal areas. Dissecting microscope photograph. X 142.
- Fig. 4. P. epiphyton foliicolous and supracuticular on S. minor in transverse section. Small space (asterisk) separates alga (A) from leaf cuticle (between arrowheads). Leaf tissue exhibits no anatomical response to presence of alga. Light microscope (LM) photograph. X 670.



- Fig. 5. Intact living P. epiphyton thallus removed from leaf surface. Dorsal view. LM photograph. X 275.
- Fig. 6. Laterally appressed branched filaments forming psuedoparenchymatous thallus in living P. epiphyton. LM photograph. X 1,520.
- Fig. 7. Abutting, non-overlapping margins of P. epiphyton thalli. Fixed material. LM photograph. X 1,480.
- Fig. 8. Immature P. epiphyton thallus from filament in senescent central region of older thallus. Living material. LM photograph. X 3,320.



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- Fig. 9. Intact pseudoparenchymatous thallus of P. epiphyton foliicolous on O. fragrans. Scanning electron microscope (SEM) micrograph. X 900.
- Fig. 10. Small P. epiphyton thallus apparently developing at senescent central region of older thallus foliicolous on O. fragrans. SEM micrograph. X 900.

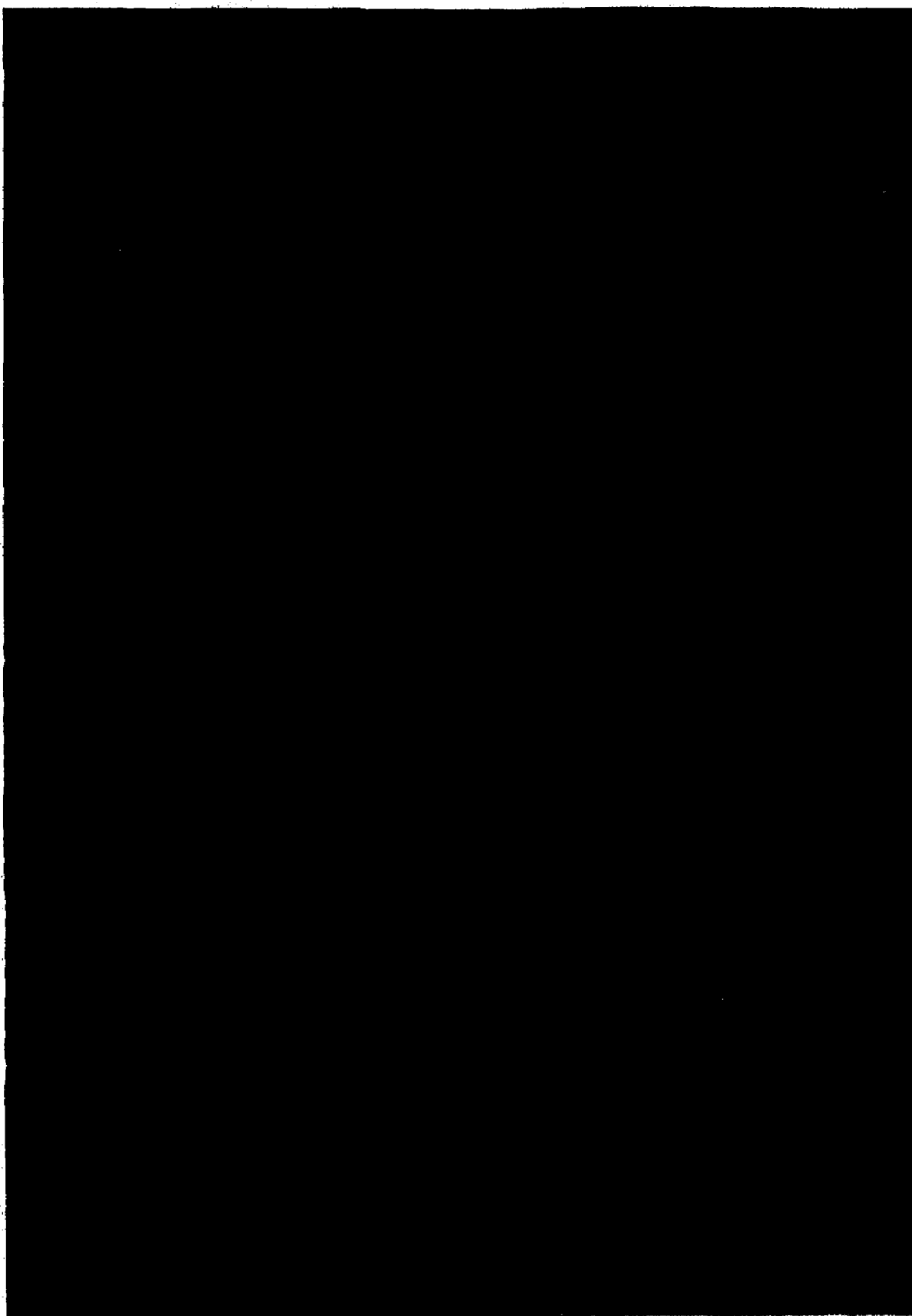


Fig. 11. Typical P. epiphyton vegetative cell in transverse section. Note nucleus (N), parietal chloroplasts (C), plastoglobuli (P), and abundant cytoplasmic haematochrome (h). Transmission electron microscope (TEM) micrograph. X 42,900.



- Fig. 12. Prominent nucleolus (Nu) in nucleus of P. epiphyton vegetative cell. TEM micrograph. X 31,000.
- Fig. 13. Mitochondria (M) and chloroplasts (C) in contact with nucleus (N) in P. epiphyton vegetative cell. TEM micrograph. X 37,000.
- Fig. 14. Chloroplast of P. epiphyton vegetative cell containing numerous starch grains (S) and plastoglobuli (P). Thylakoids do not form discrete grana. TEM micrograph CW, cell wall. X 52,800.

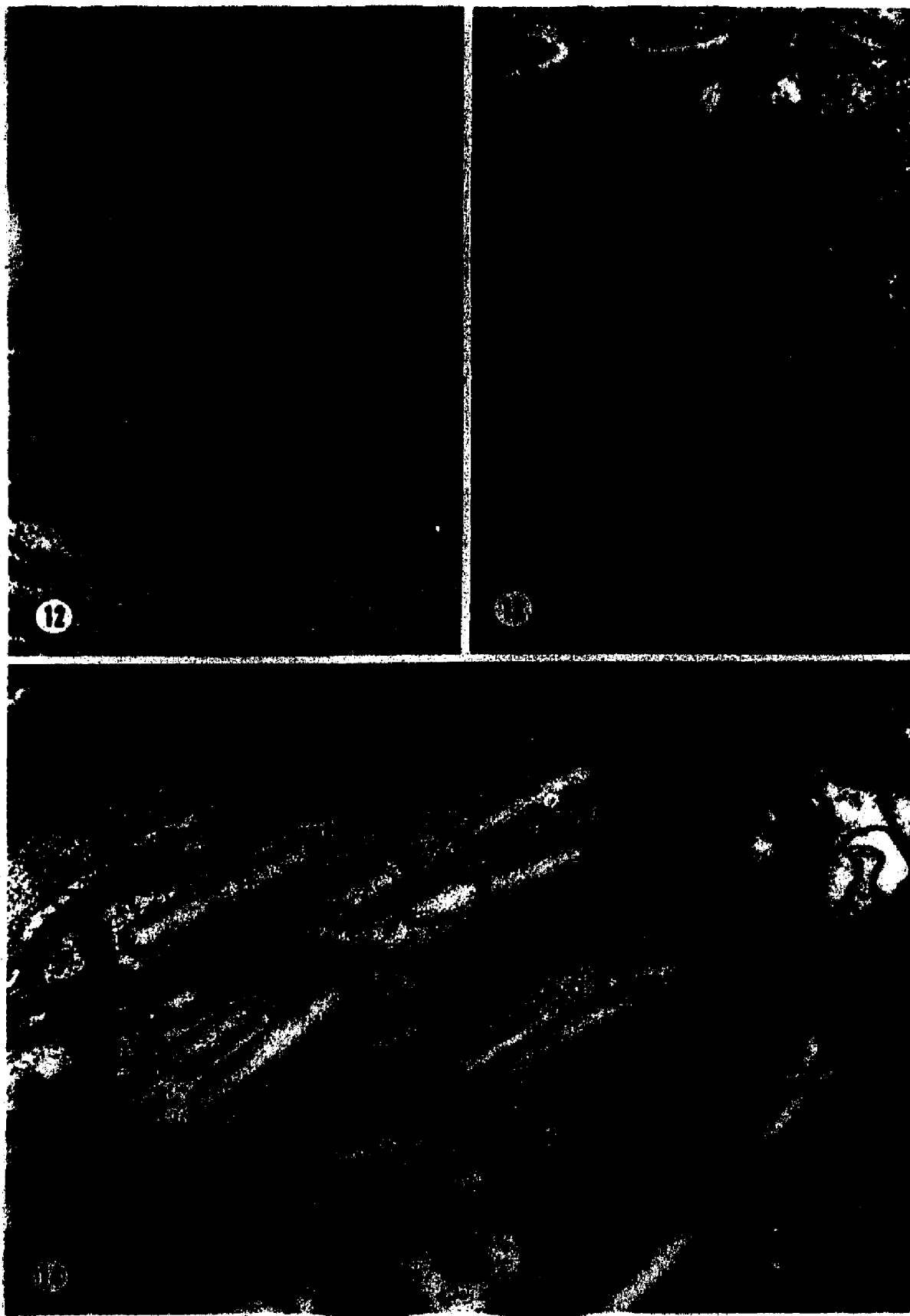


Fig. 15. Cell branching initiated by four cell wall "infolds" of P. epiphyton unicell in paradermal section. Asterisks denote wide sinus between adjacent cell wall "infolds." Note accumulation of densely stained material in the cell walls (See also Fig. 16 and 17). TEM micrograph. X 20,700.

Fig. 16. Later stage in centripetal growth of four cell wall "infolds" of P. epiphyton unicell in paradermal section. TEM micrograph. X 20,700.



Fig. 17. Recently formed septum between two cell wall "infolds" of P. epiphyton. Paradermal section. TEM micrograph. X 13,050.

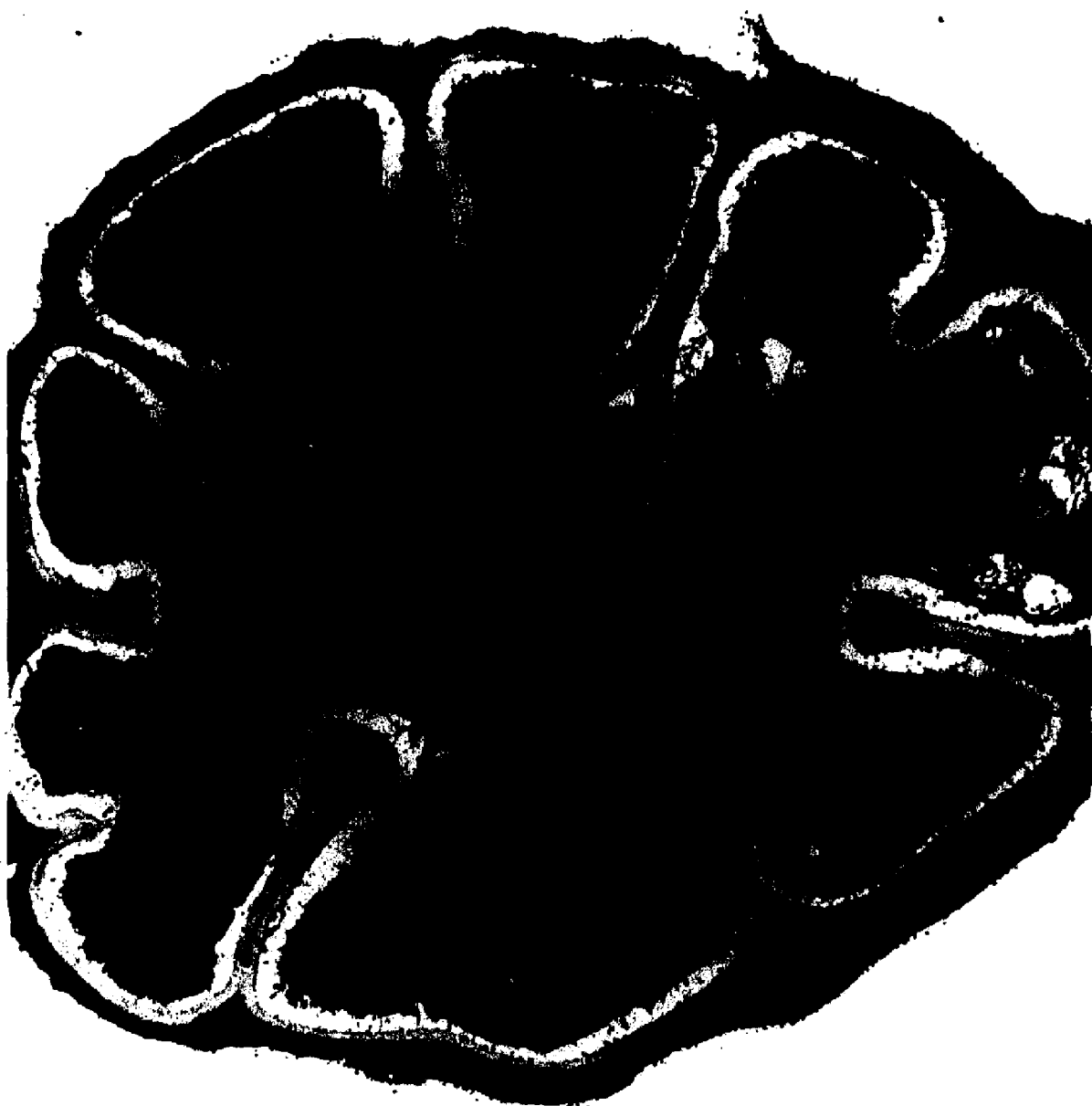
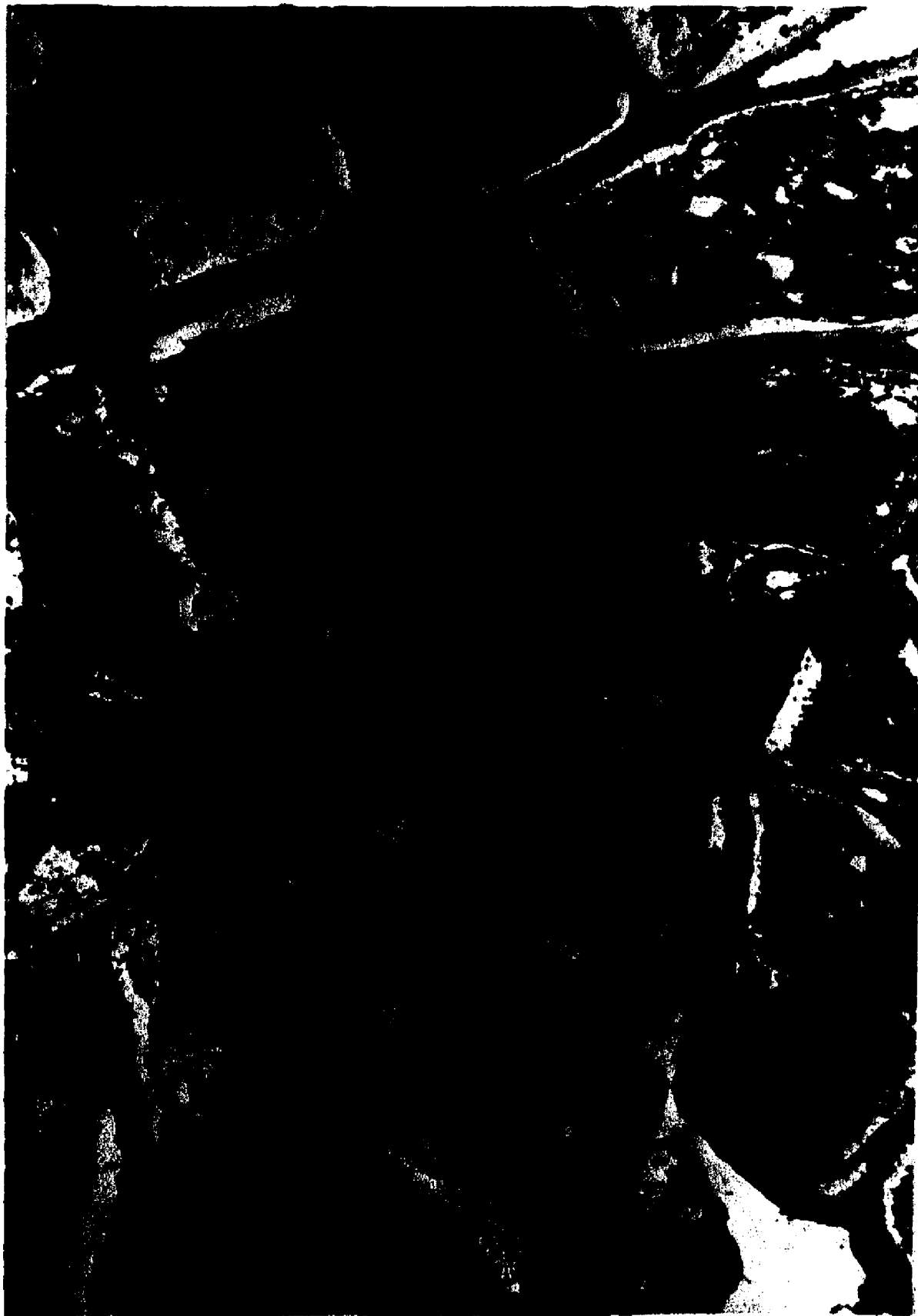


Fig. 18. Laterally appressed branching filaments forming pseudoparenchymatous P. epiphyton thalli in paradermal section. Note short cell wall "infolds" (arrows) and area of contact between thalli. TEM micrograph. X 6,000.



- Fig. 19. Marginal cell of nearly mature P. epiphyton thallus with "infolded" cell wall in paradermal section. TEM micrograph. X 14,000.
- Fig. 20. Slight plasmolysis created space (S) between cytoplasm and "infolded" cell wall of marginal cell of P. epiphyton in paradermal section. Note thickness of wall at tip of "infold." TEM micrograph. X 48,000.

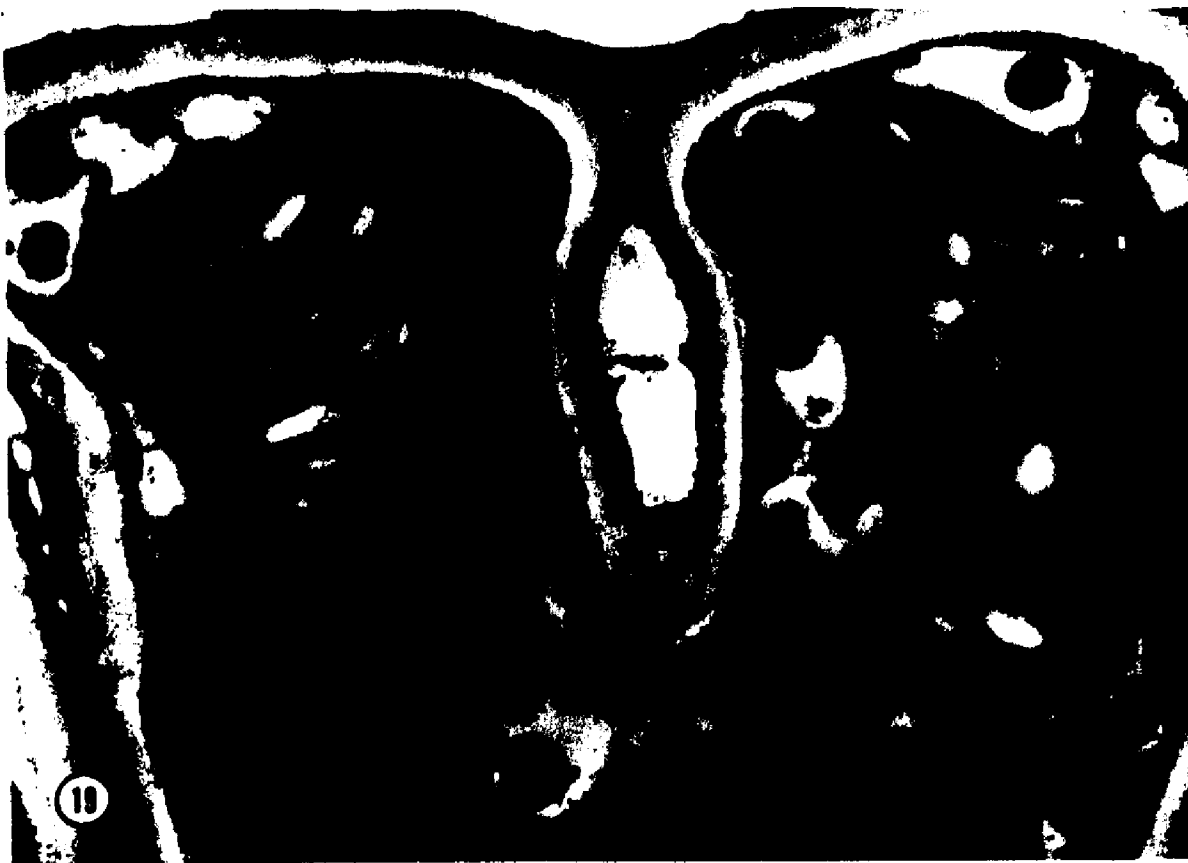


Fig. 21. One type of branching pattern in P. epiphyton in which one septum connects lateral wall (LW) with tip of "infold" (asterisk), and other septum (between arrowheads) connects other lateral wall with region slightly above "infold." Paradermal section TEM micrograph. X 20,300.



- Fig. 22. Branching pattern in P. epiphyton wherein septa connect opposite lateral walls at positions slightly distal to tip of wall "infold" in paradermal section. TEM micrograph. V, vacuole. X 7,500.
- Fig. 23. Detailed view of vacuole (V) immediately beneath tip of cell wall "infold" in paradermal section. TEM micrograph. X 39,000.

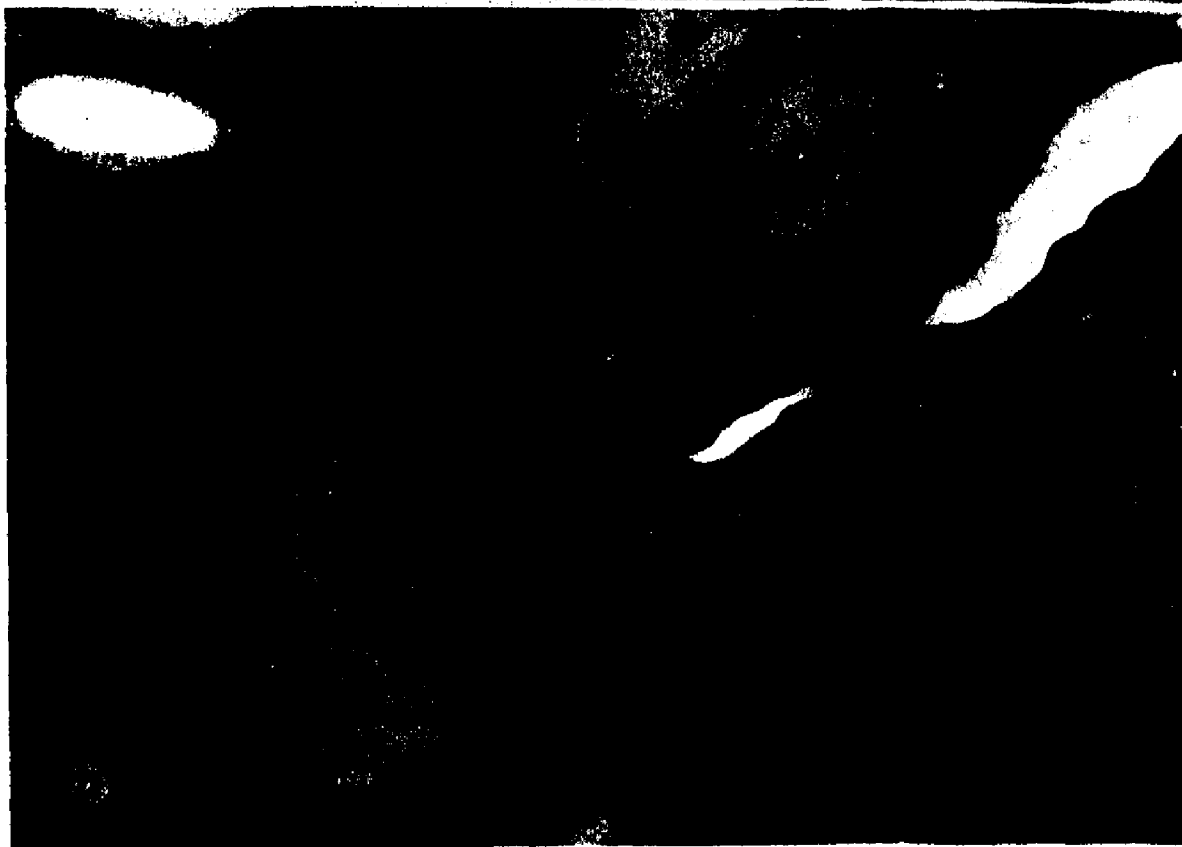


Fig. 24. Uncommon branching pattern in P. epiphyton. Cell wall "infolded" twice producing a trifurcation (asterisks denote tips of wall "infolds"). Paradermal section. TEM micrograph. X 14,500.

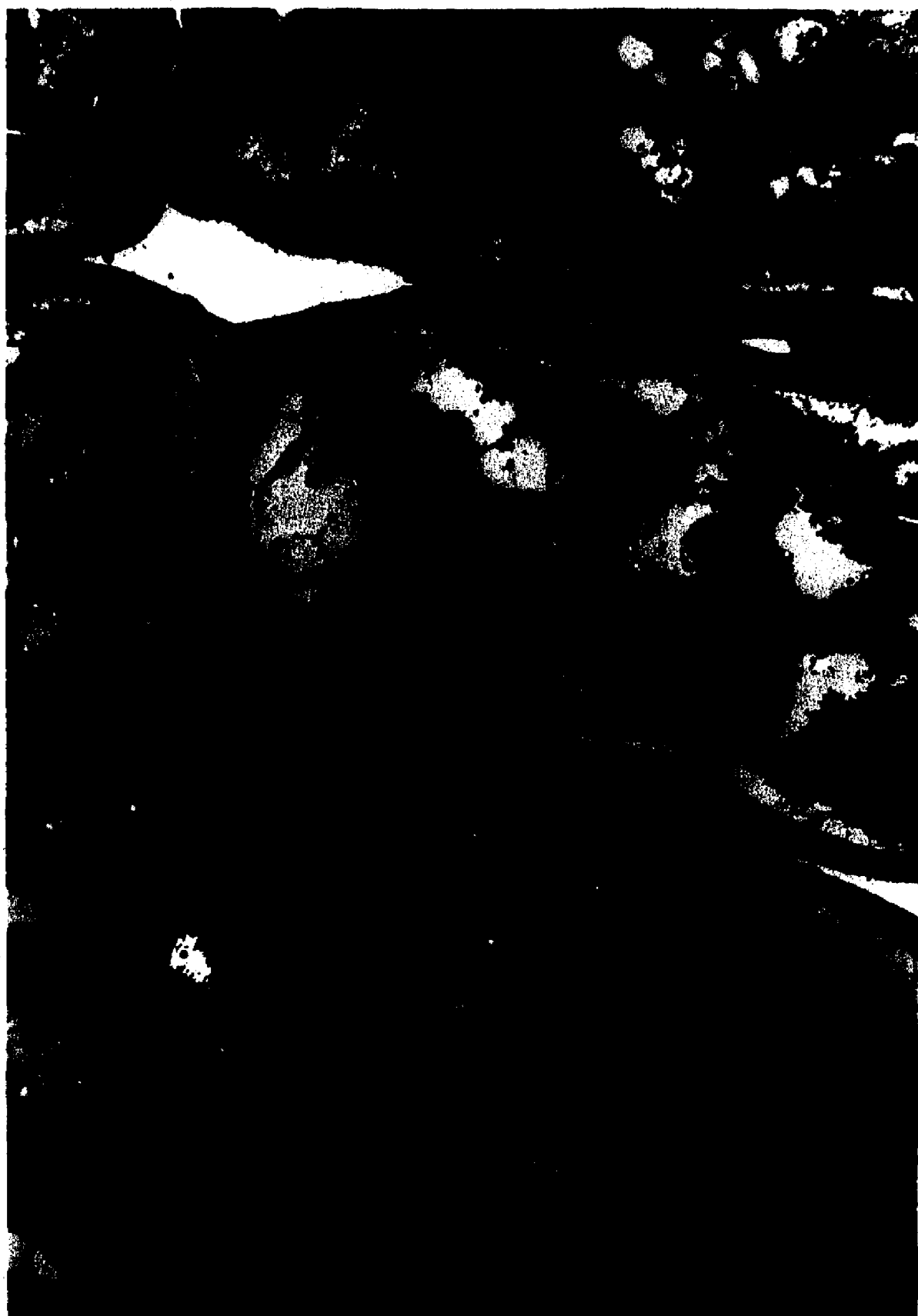


Fig. 25. Centrally located septal pit in P. epiphyton. Note plasmodesmata, bordering ring (arrows), and thinness of wall within pit area. Paradermal section. TEM micrograph. X 38,500.

Fig. 26. Centrally located septal pit in P. epiphyton. Wall within pit is approximately same size as septal wall outside pit area. Paradermal section. TEM micrograph. X 50,700.



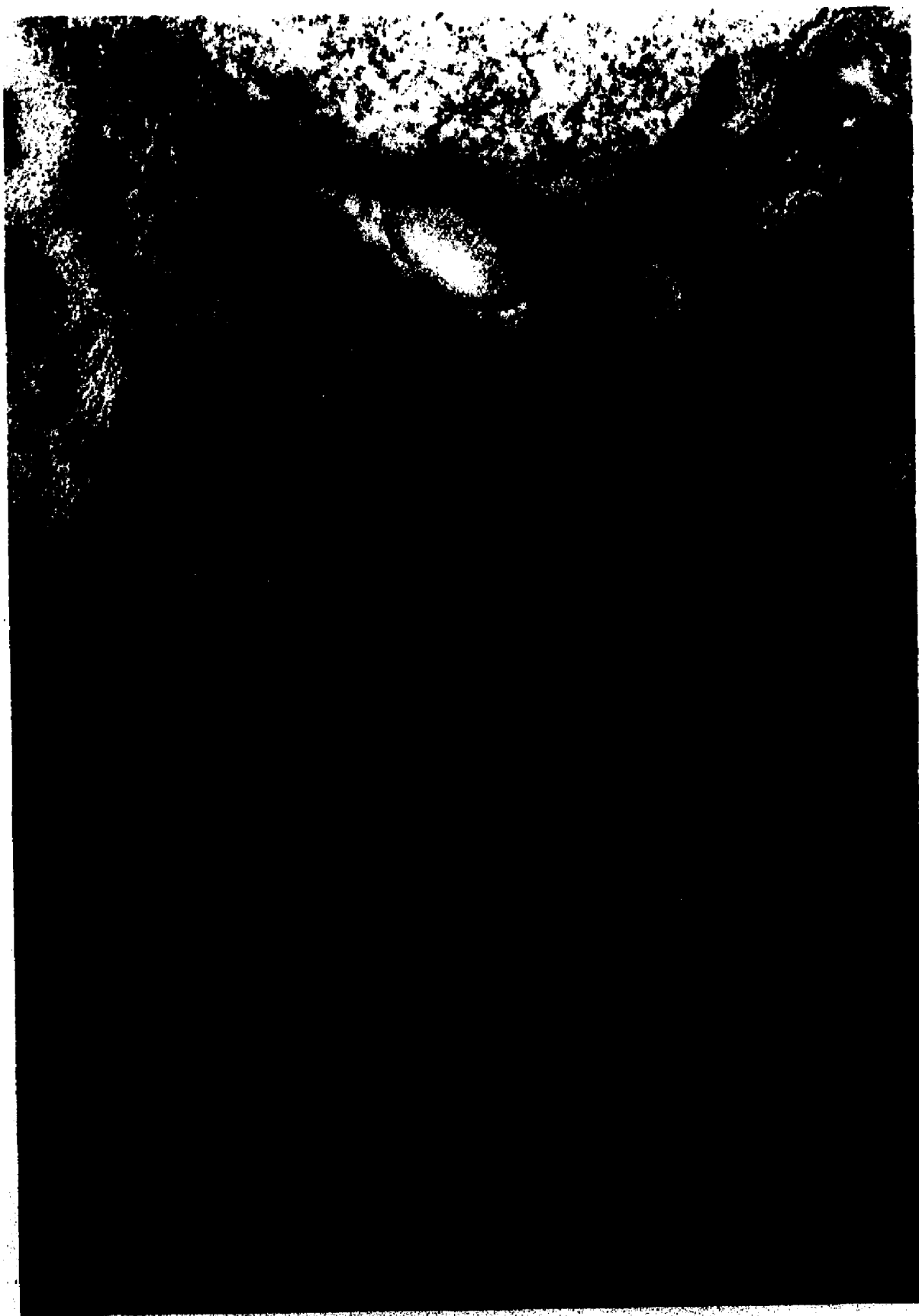
Fig. 27. Centrally located septal pit area in P. epiphyton showing localization of plasmodesmata. Oblique section. TEM micrograph. X 48,000.



Fig. 28. Centrally located septal pit area in P. epiphyton.
Note relative area of septum occupied by plasmodesmata.
Oblique section. TEM micrograph. X 20,000.



Fig. 29. Higher magnification view of centrally located pit area in *P. epiphyton*. Plasmodesmata haloed by lightly stained area of septal wall (arrowheads). Note densely stained plasmalemma (arrow) which lines plasmodesmata. Oblique section. TEM micrograph. X 69,600.



Discussion and Conclusions

Although the specimens of Phycopeltis which were collected for this study grew on adaxial leaf surfaces of seed plants, reports of Phycopeltis occurring on abaxial leaf surfaces (Allen, 1973) and also of Phycopeltis occurring on bryophytes and ferns (e.g. Suematu, 1957) make it almost certain that Phycopeltis growing in Louisiana could colonize these niches as well.

The results of the present study confirm previous reports that Phycopeltis species are foliicolous and supracuticular and do not cause an observable wounding response in plant host tissues. However, the results do not conclusively demonstrate that they are nonparasitic epiphytes. Although these algae are considered to be nonparasitic epiphytes, the physiological interactions between Phycopeltis and its plant host have not been investigated. Host specificity is definitely suggested by the observation that Phycopeltis is foliicolous on only certain plants while adjacent potential host plants are left unoccupied by the alga. In view of the work of Vidhyasekaran and Parambaramani (1971a, b) indicating that Cephaleuros depends on its plant host for certain amino acids and sugars, future investigations on the physiological interactions between Phycopeltis and its hosts are definitely warranted.

The observation that all P. epiphyton thalli are less than 1 mm in diameter is consistent with the suggestion that marginal growth of

thalli is determinate. If thallus growth were indeterminate, the maximum observed diameter would be decided by the natural life span of the alga and/or the leaf of the host plant, and environmental conditions. Thus, the "observed maximum" diameter would vary among populations of different leaves and no actual maximum diameter would exist.

Phycopeltis epiphyton contains a typical assortment of eukaryotic cellular organelles which individually do not warrant extensive comment; however, certain aspects of vegetative cell ultrastructure do require further discussion. The intimate association between mitochondria and other organelles has been observed in many plants but apparently the association is not permanent. For example, Wildman et al. (1962) reported a frequent association between mitochondria and chloroplasts. However, as explained by these authors, this was an impermanent association due to the eventual "peeling" of mitochondria from the chloroplasts. Gunning and Steer (1975) doubt that the numerous mitochondria in transfer cells are ever associated with the plasmalemma (as might be expected). In P. epiphyton, the often observed association between mitochondria and nuclei is probably temporary; however, it is entirely possible that short-term associations between mitochondria and other organelles reflect immediate energy requirements.

The chloroplasts of P. epiphyton are not unusual; however, a few comments about these organelles are appropriate because the ultrastructure of the chloroplasts in this alga has never been described. The thylakoid membranes do not form discrete grana. As

first described by Gibbs (1962), all green algae investigated have a granal association between thylakoid membranes, but discrete higher plant-type grana have never been observed.

Because only the green algae (including Prasinophyceae) store excess photosynthate in chloroplasts, it is not unusual for starch grains to occur in the chloroplasts of P. epiphyton. However, it is puzzling to read a statement by Bourrelly (1966) that starch is not produced in chroolepidaceous algae. Despite variation in size and abundance, starch grains were always present in P. epiphyton. Perhaps Bourrelly's observations should be reexamined because although rigorous chemical analyses for starch were not performed on P. epiphyton, putative starch grains were observed in P. epiphyton, Cephaleuros virescens, and Trentepohlia species. Although the possibility that the starch grain-like inclusions are not starch cannot be entirely discounted, the hypothesis that a "new" storage product exists in the Chroolepidaceae seems unnecessary at the present time. The observed absence of pyrenoids confirms earlier light microscopic reports for P. epiphyton and other chroolepidaceous taxa (e.g. Printz, 1939).

The chloroplasts also contained dense osmiophilic globules. These bodies have been termed plastoglobuli and have been reported to occur in chloroplasts of numerous other algae, as well as in archegoniate and seed plants (Lichtenthaler, 1968). Plastoglobuli are thought to be an extra-thylakoidal reservoir of excess lipids, which increase in number and/or volume as chloroplasts age or transform into chromoplasts (see e.g. Lichtenthaler, 1968; Sprey, 1970). As

with other plants, the significance of the increase in the volume and number of plastoglobuli observed in aging chloroplasts of senescing P. epiphyton cells is not understood.

The general term haematochrome has been used for the orange-red cytoplasmic (extraplastidic) lipid pigment in P. epiphyton. It is also known as euglenorhodone (Nakayama, 1962), astacin (Gilchrist and Green, 1960), and astaxanthin (see e.g. Goodwin, 1974). In Haematococcus pluvialis the pigment was shown to be a ketonic carotenoid (Goodwin and Jamikorn, 1954) and, as has been described for Haematococcus lacustris (Lang, 1968), the pigment in P. epiphyton appears to be produced and stored in the cytoplasmic ground substance.

The question of whether or not the pigment is bounded by a single membrane is intriguing. Dr. W. R. Bowen (University of Arkansas) believes that the haematochrome inclusions in Haematococcus are membrane bound (1965, and personal correspondence); however, as noted by Lang (1968), Bowen employed only a potassium permanganate (KMnO_4) fixation. Lang (1968), using both KMnO_4 and glutaraldehyde-osmium fixations, concluded that there was no membrane surrounding haematochrome inclusions. The observations on P. epiphyton as well as those on Cephaleuros and Trentepohlia (Dr. R. L. Chapman, unpublished results) support the idea that in general, haematochrome inclusions are not membrane bound.

There are suggestions that the development and accumulation of haematochrome is in response to environmental factors (i.e. light quality and intensity) and unspecified physiological states of the cell (e.g. King, 1954, 1955; Lang, 1968; Prescott, 1968). As in

Cephaleuros and Trentepohlia, high light intensity apparently affects haematochrome production in P. epiphyton because the algal thallus in shady habitats frequently is grass-green in color (due to chlorophyll) while in exposed habitats it is orange-red. The orange-red color of the thallus is due to the large amount of haematochrome masking the color of the chlorophyll.

Although the cellular organelles, storage products, and certain pigments of P. epiphyton are not particularly unusual, the process of cell branching is intriguing. Cell branching is initiated by a terminal cell wall infolding, not by a radial division as described by Thomas (1913). One can propose at least two distinct modes of terminal cell wall infolding. First, an area of the terminal cell wall could actually infold centripetally forming a characteristic "loop." However, such an explanation does not seem satisfactory because the cell wall is a rather rigid non-pliable structure that would not lend itself well to forming "loops." Second, a specific area of the terminal cell wall could become inactive (no growth) while the parts of the cell adjacent to this inactive region could continue to grow producing the apparent infold. This alternate hypothesis is more attractive because an "infolding" of the cell wall per se is not postulated and because a vacuole was frequently observed at the postulated inactive area (see Fig. 23). This vacuole could be involved in inhibiting cell wall growth in the region in which it is located; however, the mode of this inhibition (if it does occur) remains to be elucidated.

After initiation of cell branching, septa form between lateral cell walls and either the tip of the terminal cell wall infold or the areas immediately above the tip of the infold. Precisely how septal formation occurs in P. epiphyton is not clear because despite extensive study, the sequence of septal formation was not observed. Nevertheless, indirect evidence is available. In other algae, septal formation occurs by infurrowing, cell plate formation, or a combination of both infurrowing and cell plate formation. Those algae that possess the infurrowing type of septum formation do not contain plasmodesmata in their cross walls, while algae that possess the cell plate type of septum formation and those algae that form septa by a combination of infurrowing and cell plate formation can contain plasmodesmata in cross walls (e.g. Fowke and Pickett-Heaps, 1969a, b; Floyd, 1971; Floyd et al., 1972a, b; Mattox et al., 1972). Although Stewart et al. (1973) believe that there is little doubt that cell plates are both ontogenetically and phylogenetically associated with the presence of plasmodesmata, it should be noted (as pointed out in part by Stewart and co-workers, 1973) that in certain plants other than algae, the presence of a cell plate is not necessarily related to the presence of plasmodesmata (Burgess, 1971). In addition, Robards (1975) points out that plasmodesmata can possibly form secondarily (i.e. penetrating a mature cell wall). However, because the septa of P. epiphyton do contain plasmodesmata, the formation of the septum (or the central area of the septum, i.e. the pit area) via a cell plate is strongly indicated. Further, limited observations on incomplete cross walls and descriptions of septal formation by early

workers (e.g. Millardet, 1870; Thomas, 1913; Printz, 1939), indicate that infurrowing also is most certainly involved in septal formation (Chapman and Good, 1978).

In addition to observations on incomplete septum formation and earlier light microscopic reports, the morphology of the central pit area of the septum also strongly suggests that both infurrowing and cell plate formation are involved in septal formation. A septum with a central pit area containing plasmodesmata could be formed by either of two entirely different methods. In the first method, the central localization of plasmodesmata occurs by selective loss of plasmodesmata from the outer regions of the septum [similar to the formation of primary pit fields in vascular plants (e.g. Burgess, 1971; Stewart et al., 1973; Chapman and Good, 1978; Chappell et al., 1978)]. The second method is the formation of a cell plate in the central area amid a centripetally infurrowing septum.

Observations on numerous newly formed walls in P. epiphyton argue against the selective loss of plasmodesmata because plasmodesmata have never been observed in outer regions of the septa. The evidence available strongly suggests the septa of P. epiphyton have been produced by an infurrowing septum accompanied by cell plate formation in the central area.

The other chroolepidaceous algae, Cephaleuros and Trentepohlia, lack the ring of lightly stained material and the differentially thickened septum usually present in P. epiphyton. Although as noted by Chapman and Good (1978), it is unlikely the septa of both Cephaleuros and Trentepohlia are formed in exactly the same manner

as P. epiphyton, a similar combination of infurrowing and cell plate formation is certainly possible. Further, many ultrastructural studies render unjustifiable the implicit analogy between the central pit region of the Chroolepidaceae with the pit connections of red algae and the pit connections, microplasmodesmata, pore or heterocyst pore channels in the blue green algae (see Chapman and Good, 1978, for details). In addition, the central pit-like connection in the aquatic genus Ctenocladus (Blinn and Morrison, 1974) is so unlike the central pit region found in the chroolepidaceous algae that Ctenocladus should not be included in the Chroolepidaceae as some authors have proposed (see General Introduction).

The plasmodesmata contained in the septal central pits also warrant comment. As noted earlier, plasmodesmata between vegetative cells of P. epiphyton have a diameter of approximately 40-50 nm and lack a desmotubule (central core) which is sometimes present in the plasmodesmata of vascular plants as well as green algae (e.g. Burgess, 1971; Floyd, 1971; Stewart et al., 1973). The suggestion by Stewart et al. (1973) that a desmotubule is always formed in new plasmodesmata but does not always persist is interesting and could explain the apparent absence of desmotubules in Cephaleuros, Phycopeltis, and Trentepohlia. Further, peripheral subunits which were reported in the plasmodesmata of Bulbochaete (Fraser and Gunning, 1969) are not present in the plasmodesmata of the chroolepidaceous algae.

Although it is probable that the chroolepidaceous septal formation occurs via both infurrowing and cell plate formation, the question remains whether a phragmoplast or phycoplast system of

microtubules is involved in the cytokinetic process. In recent years, two separate and distinct types of cytokinetic microtubular arrangements have been described in algae (e.g. Pickett-Heaps, 1969; Pickett-Heaps and Marchant, 1972; Stewart and Mattox, 1975). In the phragmoplast type of microtubular arrangement (classically described in land plants and found in only certain green algae), microtubules are arranged perpendicular to the plane of cell division; while in the phycoplast microtubular arrangement (not described in land plants and found in certain green algae) microtubules parallel the plane of cell division. Further, the possibility exists that a phragmoplast or a phycoplast could be associated with a cell plate, an infurrowing, or both a cell plate and an infurrowing type of cytokinesis (e.g. Fowke and Pickett-Heaps, 1969b; Stewart *et al.*, 1973; Chapman and Good, 1978; Chappell *et al.*, 1978). In the proposed green algal evolutionary line to land plants (Charophyceae, *sensu* Stewart and Mattox, 1975) cell plate formation (as in land plants) is an integral part of a phragmoplast microtubular arrangement. Which cytokinetic microtubular arrangement (phragmoplast or phycoplast) occurs in the vegetative cells of Cephaleuros, Phycopeltis, and Trentepohlia remains an important question.

The vegetative ultrastructure of P. epiphyton has been described for the first time. Further, the specialized septa between vegetative cells reported in this study are unique among all algae. In general, the information obtained has facilitated comparisons not only between Phycopeltis and other chroolepidaceous algae, but also among the Chroolepidaceae and certain other green algae as well.

Chapter II

Sporopollenin in the Cell Wall

Introduction

A densely staining material (DSM) was observed in the cell walls of Phycopeltis epiphyton. This chapter presents chemical and ultrastructural data indicating that this material is a sporopollenin and discusses its adaptive significance in this and other algae (see also Good and Chapman, 1978). The successful identification of the sporopollenin not only further characterizes P. epiphyton but also provides a basis for comparison with other algae in the same or different families.

The selection of experimental approaches for identifying the DSM was primarily guided by two observations. First, the foliicolous subaerial habitat of P. epiphyton suggested that the DSM might afford protection (e.g. against desiccation). Second, the report of Kirchheimer (1942) that P. microthyrioides was found with pollen grains in Tertiary brown coal deposits suggested that the unknown material might be chemically inert and relatively resistant to degradation. Because sporopollenins are some "of the most extra ordinarily resistant materials known in the organic world" (Faegri and Iversen, 1964), it was decided initially to test for sporopollenins by the method of acetolysis. The presence of these substances in algae is not without precedent. Sporopollenins have been reported to occur in species of six genera of green algae: Chlorella, Scenedesmus, Pediastrum, Chara, Prototheca, Coelastrum (Brooks and Shaw, 1971; Atkinson et al., 1972; Pickett-Heaps and Staehelin, 1975; Marchant,

1977) and according to Atkinson and co-workers (1972), perhaps in the dinoflagellate Pyrocystis studied by Swift and Remsen (1970).

Materials and Methods

Transmission Electron Microscopy

See Chapter I (Materials and Methods).

Chemical and Spectrophotometric Analyses

Acetolysis-Algal thalli (ca. 40-200 per tube) were placed in 12 ml centrifuge tubes containing 5 ml of glacial acetic acid and centrifuged. The acid was removed after 5-10 min and acetolysis mixture (9:1 vol/vol acetic anhydride: conc. sulfuric acid) was cautiously added to the tubes. The tubes were placed in a water bath (23 C) and the water was brought to boiling (99-100 C) for 5-30 min. The tubes were removed from the water bath and centrifuged, the supernatant was decanted, and the remaining algal material was rinsed with glacial acetic acid (2X) and washed with water (2X). Some of this acetolysed material was dehydrated and prepared for transmission electron microscopy (as previously described).

Chemical degradation-Degradation of algal thalli was attempted either with an aqueous solution of 30% (wt/vol) chromic acid at room temperature (ca. 23 C) or with 2-aminoethanol (ethanolamine) for 3 hr at 97 C (Bailey, 1960) or for 3 hr at 155 C (Rowley and Flynn, 1966). Fresh P. epiphyton was also stained with dilute solutions (pH 7.0) of Primuline (Eastman) and observed with a Leitz Orthoplan microscope fitted with a high pressure mercury lamp (HBO-200W), an oil immersion dark field condenser, and the following filters: A BG 38 heat

absorbing filter, a UGI excitation filter, and K430 barrier filter (Waterkeyn and Bienfait, 1971). The methods described by Jensen (1962) were followed in testing for lignin (phloroglucinol procedure), lipids (Sudan IV procedure), chitin, and cellulose. Lignin extraction followed the procedure of Stafford (1960). In addition, the test for cellulose was performed on Magnolia grandiflora pollen grains.

Infrared absorption spectrophotometry—Fresh P. epiphyton thalli and pollen grains of M. grandiflora were separately prepared as follows: plant material was acetolysed for 15 min (see above), rinsed (2X) with 0.02 M potassium phosphate buffer (pH 6.8), and boiled for 10 min in 1 N sodium hydroxide. After centrifugation, this material was rinsed in buffer, distilled water, absolved methanol (2X), and placed in ether. The material was incorporated, in vacuo, into potassium bromide pellets and analysed in Perkin Elmer 137 and 601 infrared spectrophotometers.

Observations

The cell walls of P. epiphyton contained two zones, a lamellated outer zone (OZ) which was formed by a densely staining material (DSM) and an inner zone (IZ) that was also lamellate but less densely stained (Fig. 30). The thickness of the outer zone was variable, but consistently was greatest on the upper surface of the thallus where it resembled a "cuticle." The lamellated appearance of the cell wall resulted from electron transparent lines within the DSM. The thickness of the electron transparent lines ranged from approximately 4 to 11 nm. In some homogeneous areas of the outer wall zone no electron transparent lines were visible.

An acetolysed algal thallus (Fig. 31) retained its disc-like shape, integrity, and fastigiate arrangement of filaments. Also, the average thickness of acetolysed cell walls (e.g. Fig. 32) was basically unchanged from that of untreated material.

The results of different chemical tests and stains performed on fresh P. epiphyton are shown in Table 1. Chromic acid caused dissolution of both fresh and acetolysed algal thalli after 5-15 min, while ethanolamine did not cause any apparent change in the cell walls of fresh specimens. Secondary fluorescence occurred in the cell walls of fresh material stained with the fluorochrome, Primuline, and preliminary tests for lignin, chitin, and lipids in vegetative cell walls of P. epiphyton were negative. In addition, light

Table I. Results of chemical tests and staining on Phycopeltis epiphyton.

Test	Reaction
Chromic acid	Thallus dissolution
Ethanolamine	No observable change
Primuline	Fluorescence
Phloroglucinol	No observable change
Lignin extraction	No observable change
Sudan IV	No observable change
Chitin-Chitosan	No observable change
IKI-65% H_2SO_4	Orange color (negative for cellulose)
Zinc-chlor-iodide	Orange color (negative for cellulose)

microscopic inspection after lignin extraction showed an intact thallus with no observable change in cell walls. In the test for cellulose, walls of both P. epiphyton thalli and M. grandiflora pollen grains stained orange (negative for cellulose).

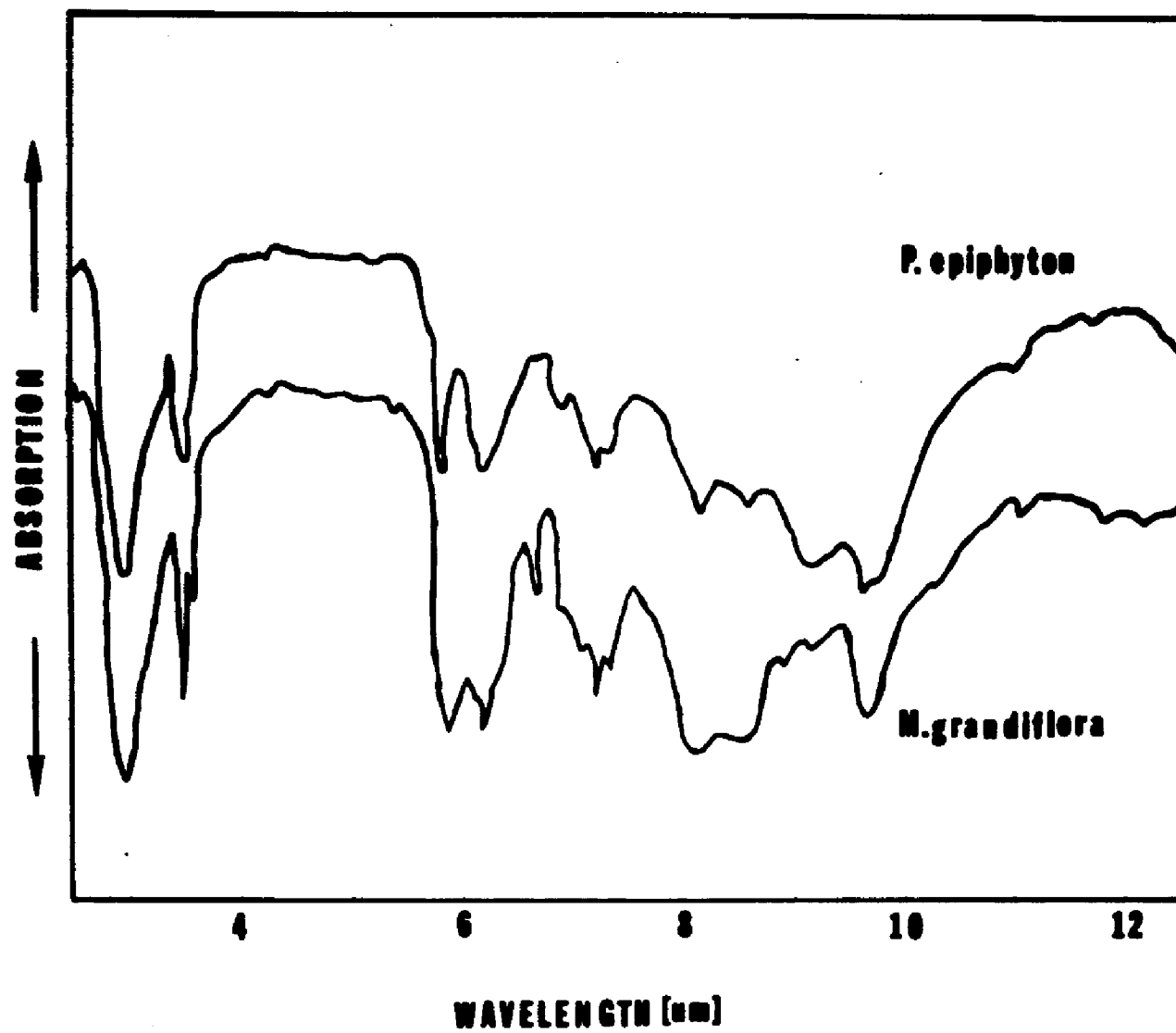
The infrared absorption spectra of acetolysed P. epiphyton and acetolysed M. grandiflora pollen grains are shown in Figure 33.

- Fig. 30. Electron transparent lines surrounded by densely staining material create lamellated appearance in both zones of Phycopeltis epiphyton cell wall. Transverse section. Transmission electron microscope (TEM) micrograph. OZ, outer zone; IZ, inner zone. X 103,600.
- Fig. 31. Remains of acetolysed P. epiphyton. Algal thallus retained its disc shape and filamentous arrangement. Light microscope photograph. X 912.
- Fig. 32. Lateral wall of acetolysed P. epiphyton. Note lamellated appearance of wall is still evident. Paradermal section. TEM micrograph. X 66,000.



Fig. 33. Infrared absorption spectra of acetolysed Magnolia grandiflora pollen grains and acetolysed P. epiphyton thalli.

③



Discussion and Conclusions

Chemical and Spectrophotometric Analyses

The data indicate that the densely staining material (DSM) present in the walls of P. epiphyton is a sporopollenin. Sporopollenins typically form the exines of pollen grains as well as the walls of many archegoniate land plant spores (e.g. Horner et al., 1966; Rowley and Southworth, 1967; Heslop-Harrison, 1968a; Stetler and DeMaggio, 1976), and as previously mentioned (see Introduction), sporopollenins have been reported in certain species of six green algal genera. Sporopollenin is a unique class of substances, resistant to biological and chemical nonoxidative degradation and thought to be an oxidative polymer or copolymer of carotenoids and/or carotenoid esters (Brooks and Shaw, 1968, 1971; Shaw, 1971). Southworth (1974) accepted this characterization, but also indicated that phenolic groups occur in native sporopollenins. Resistance to acetolysis has been used as a microchemical test for sporopollenins (Heslop-Harrison, 1968b) because it is "the only organic constituent of plant cell walls that withstands acetolysis for more than a few minutes" (Atkinson et al., 1972) and "the only class of plant wall polymer to survive this treatment without solvation or radical structural changes" (Heslop-Harrison, 1971a). The exact chemical reactions occurring in organic material being acetolysed are not known and perhaps the closest chemical definition of acetolysis would be a "simultaneous acetylation and hydrolysis" (Brown, 1960).

The resistance of P. epiphyton walls to acetolysis is a strong indication that a sporopollenin is present. In fact, resistance to acetolysis has been used by some investigators as the sole criterion for the identification of sporopollenins in plants. However, it has also been suggested that other (unspecified) organic materials survive this treatment (Skvarla and Rowley, 1970) and, more specifically, that lignin can survive acetolysis (Southworth, 1974).

The possibility that substances other than sporopollenins could survive acetolysis, necessitated the additional chemical tests and stains which further substantiated the results of acetolysis (see Table 1). The digestion by chromic acid supports the hypothesis that a sporopollenin is present in P. epiphyton since, with one exception (*viz.*, Ambrosia trifida pollen grains, Southworth, 1974), sporopollenins are susceptible to oxidation by this acid. Further, the complete oxidation of thalli by chromic acid clearly indicates that silica, which can withstand oxidation by chromic acid as well as acetolysis, is not a major cell wall component of P. epiphyton as has been reported in the sporopollenin-containing alga Pediastrum and certain pollen grains (Millington and Gawlik, 1967; Crang and May, 1974). The insolubility of P. epiphyton in ethanalamine is difficult to interpret because this reagent has given highly variable results in a number of different pollen investigations (*e.g.* Bailey, 1960; Southworth, 1974).

The secondary fluorescence of P. epiphyton cell walls stained with Primuline is a third indication of the presence of a sporopollenin. Although lignin also can cause this secondary fluorescence

(Waterkeyn and Bienfait, 1971), negative phloroglucinol tests and lignin extractions indicate that lignin is not present in P. epiphyton. The apparent absence of lignin in P. epiphyton further supports the hypothesis that Primuline-induced secondary fluorescence in P. epiphyton is caused by a sporopollenin and that lignin is not the material resisting acetolysis.

The microchemical tests for cellulose (IKI-65% H_2SO_4 and zinc-chlor-iodide) in P. epiphyton thalli and M. grandiflora pollen grains produced an orange color rather than the blue color indicative of cellulose. Such yellow to orange staining can indicate the presence of large amounts of chitin, lignin, suberin, or cutin (Jensen, 1962); however, failure of P. epiphyton cell walls to take up Sudan IV (a stain which depends on the solubility of the dye in lipid) indicates that there are no large amounts of suberin and cutin. Further, neither lignin nor chitin is present in P. epiphyton and thus the orange color reaction in P. epiphyton walls is not caused by these substances. In view of these data, it is likely that the sporopollenin stains orange and may prevent the staining of cellulose. Southworth (1974) came to a similar conclusion after studying Ambrosia trifida pollen grains.

The infrared absorption spectra of acetolysed P. epiphyton thalli and M. grandiflora pollen grains (Fig. 33) are comparable to infrared absorption spectra of sporopollenins from other green algae, pollen grains, and spores of lower archegoniate land plants, as well as a synthetically produced sporopollenin (Brooks, 1971; Brooks and Shaw, 1971; Atkinson et al., 1972). These spectra are by themselves

strong evidence for the presence of a sporopollenin and, combined with the results of microchemical tests, convincingly reinforce the conclusion that a sporopollenin is present in P. epiphyton.

Fine Structure

Electron transparent lines of the DSM are part of both the inner and outer zones of lateral and terminal cell walls, and are also in the cross walls of P. epiphyton. Similar electron transparent lines have been observed or described in exines which contain sporopollenins of many pollen grains and spores of archegoniate land plants (e.g. Larson and Lewis, 1961; Rowley, 1962; Dickinson and Heslop-Harrison, 1968). In discussing these electron transparent lines or lamellae, the terminology of Dickinson and Heslop-Harrison (1971) is employed. A "sporopollenin lamella" is a tripartite structure which is a "membrane" with sporopollenin deposited (polymerized?) on its two surfaces. The "parent lamella" is the 4-5 nm (sometimes less) thick, central transparent zone of the sporopollenin lamella, and, as pointed out by these investigators, the parent lamella corresponds to the "white line" described in the exine of Ipomoea purpurea pollen grains by Godwin et al. (1967) and also to the "low dense line" of Rowley and Dunbar (1967). Presumably, the lamellated aspect of the cell walls of P. epiphyton is caused by an abundance of sporopollenin lamellae each with an inner parent lamella (the electron transparent line). Although the composition of parent lamellae is unknown, freeze-fracture has revealed that the parent lamella is a "real discontinuity" (Southworth and Branton, 1971). According to Dickinson

and Heslop-Harrison (1971) the origin of parent lamellae in Lilium longiflorum is located at the plasmalemma surface. Presumably the parent lamellae of P. epiphyton also originate at this site.

Dickinson and Heslop-Harrison (1971) also have suggested that parent lamellae may be "flow pathways" for sporopollenin precursors, that is, surfaces for sporopollenin polymerization and/or deposition. A similar function would not be inconsistent with observations on P. epiphyton, but the data do not provide evidence to support or challenge this hypothesis.

Although the fine structure of P. epiphyton cell walls is in some ways quite similar to that of many pollen grain exines and spores of archegoniate plants, it is quite different from that found in other green algae that have been shown to contain sporopollenins. A "trilaminar (sporopollenin) sheath" surrounds the acetolysed cells in certain species of Chlorella, Pediastrum, Scenedesmus, and Coelastrum (Millington and Gawlik, 1970; Atkinson *et al.*, 1972; Staehelin and Pickett-Heaps, 1975; Marchant, 1977). This trilaminar sheath may be compared to a single, continuous "sporopollenin lamella" which consists of two densely staining layers separated by an electron transparent lumen (*cf.*, a "parent lamella"). In Chlorella, Coelastrum, and Scenedesmus, the trilaminar sheath is formed by a fusion of smaller, similar structures called "trilaminar plaques" which originate at the plasmalemma surface.

Functions

Sporopollenins presumably protect pollen grains against desiccation (Heslop-Harrison, 1971b) and one could suggest that a sporopollenin has a similar function in P. epiphyton. The fact that a sporopollenin is the "densely staining adhesive material" in Scenedesmus and most likely, in Pediastrum as well (Pickett-Heaps, 1975a), suggests that a sporopollenin, alone or with other compounds may be involved in adhesion of P. epiphyton to its hosts and is certainly involved in adhesion between laterally appressed branched filaments of the pseudoparenchymatous thallus.

Despite suggestions that sporopollenins protect against fungal and/or bacterial parasitism (Prescott, 1970; Heslop-Harrison, 1971b), it has been reported that sporopollenins do not prevent Scenedesmus from being parasitized (Schnepf et al., 1971a, b). Although it is not known if the cell wall of P. epiphyton protects it against parasitism, the fact that intracellular penetration of viable algal cells by fungal haustoria was never observed even though the thalli were often associated with fungal hyphae could be interpreted as support for this hypothesis.

In summary, it should be noted that although other substances besides sporopollenins can apparently survive acetolysis, some investigators (e.g. Marchant, 1977) have used acetolysis resistance as their sole criterion for suggesting and/or "establishing" the presence of sporopollenins. The present investigation utilized acetolysis, several different microchemical tests, as well as infrared absorption spectrophotometric analyses to characterize the DSM in the

cell walls of P. epiphyton. The results of these tests most certainly show that the cell walls of P. epiphyton contain a sporopollenin and the tests themselves are together the appropriate means of firmly establishing the presence of a sporopollenin in an alga.

Chapter III

Observations on the Ultrastructure of Zoosporangia, Gametangia, and Motile Cells

Introduction

This chapter records observations on the ultrastructure of reproductive structures and motile cells of Phycopeltis epiphyton, including zoosporangial development and abscission, zoospores, gametangia, and gametes. These observations will be compared to published accounts for chroolepidaceous algae, other green algae, and archegoniate plants. There are no published reports on the ultrastructure of Phycopeltis zoosporangia, gametangia, or motile cells, although there are reports on the fine structure of reproductive structures and/or motile cells of Cephaleuros (Chapman, 1976) and Trentepohlia (Graham and McBride, 1975). Except for a few line drawings, (e.g. Möbius, 1888; Millardet, 1870; Printz, 1939) the reproductive structures and motile cells of Phycopeltis have not been described heretofore.

In the presentation of observations on the ultrastructure of reproductive structures and motile cells of P. epiphyton, a knowledge of the alga's life cycle is fundamentally important, if only for correctly naming and discussing these structures. Thompson (1959) observed a dwarf sporophyte (with microzoosporangia) in the life cycles of both Cephaleuros and Stomatochroon, and thereby raised the possibility that a similar stage exists in the life cycles of Phycopeltis and Trentepohlia as well. Regardless of whether or not a dwarf sporophyte generation exists in the life cycle of Phycopeltis,

the terminology employed in this chapter is correct because the identification and naming of the basic reproductive structures (viz. zoosporangia and gametangia) are in no way affected by the possibility.

Materials and Methods

Transmission Electron Microscopy

Fertile Phycopeltis epiphyton was collected and prepared for thin-sectioning as described in Chapter I. Reproductive motile cells to be negatively stained were collected by wetting fertile thalli with distilled water and transferring drops of this water containing motile cells to surfaces of either Parafilm "M" (Marathon Products) or paraffin. Parlodion-coated grids were passed through these drops and set aside until almost dry. Motile cells were fixed in situ (15-30 min) by adding a drop of 4% glutaraldehyde in 0.02 M potassium phosphate buffer (pH 6.8). The fixative was drawn-off with absorbent paper, a drop of 2% aqueous uranyl acetate was added and allowed to evaporate. Samples were observed and photographed as previously described.

Scanning Electron Microscopy

Fertile thalli were collected and prepared for scanning electron microscopy as previously described (see Chapter I). Motile cells were collected (see above) and processed either by filtration or centrifugation. In the former technique, drops of distilled water containing motile cells were transferred to a 0.20 μ m Metrice1 GA8 filter (Gelman) held in a filtration flask under slight vacuum. Motile cells were fixed in situ with 4% glutaraldehyde in 0.02 M potassium phosphate buffer (pH 6.8), rinsed with buffer and distilled water (2X and 3X,

respectively), rapidly dehydrated with acidified 2,2-dimethoxypropane (Muller and Jacks, 1975) and placed in amyl acetate. This material was critical point dried in a Denton DCP-1 critical point dryer. Small portions of filter were mounted on stubs, coated and observed as described in Chapter I. Centrifugation processing consisted of collecting motile cells (see Transmission Electron Microscopy) and transferring approximately 9 ml of distilled water containing motile cells to a centrifuge tube. The motile cells were fixed (15-30 min) by the addition of phosphate buffered glutaraldehyde (ca. 2 ml), centrifuged, rinsed, rapidly dehydrated, and placed in amyl acetate. Solution containing motile cells was transferred to Metrical GA8 filters (Gelman) and prepared for scanning electron microscopy as previously described.

Observations

Zoosporangia - External Morphology

Terminal zoosporangia borne on single-celled pedicels were present on the dorsal surface of Phycopeltis epiphyton at all times of the year, but were more abundant during the months of April through September. The zoosporangia on individual thallus were at different developmental stages (Fig. 34), indicating asynchronous initiation and/or maturation. The pedicels bearing terminal zoosporangia were produced in all areas of the thallus dorsal surface.

Numerous observations on zoosporangia at different ontogenetic stages have been placed in a logical sequence illustrating zoosporangium maturation and abscission (Fig. 35-44). Early stages in pedicel initiation were not observed. Terminal zoosporangium (Z) development was initiated at the apical end of a pedicel (P in Fig. 35). Frequently, this pedicel apical area (i.e. the immature zoosporangium) appeared rugulose.

A septal ridge (arrows) demarcated the pedicel from its rugulose apical end and indicated that at least partial development of the modified cross-wall between the zoosporangium and the pedicel (termed the zoosporangium-pedicel septum, hereafter, ZPS) had occurred. A torn portion of outer septal wall material revealed the presence of a ZPS (between arrowheads). With continued zoosporangial maturation, the surface appeared smooth (Fig. 36), the volume of the terminal

sporangium had increased, and small papillose accretions (arrows) had formed on the pedicel (Fig. 37). As the zoosporangium assumed its mature shape (Fig. 38), a single nipple-like protuberance (arrow) was formed at either a terminal, subterminal, or lateral position on the zoosporangium, but never at a sublateral position. Such nipple-like protuberances were, at later stages, escape pores (Fig. 39-40, 42). In enlarged zoosporangia which contained well developed, plugged escape pores, the first visible event of abscission was a tear in the outer septal (lateral) wall (Fig. 39 arrowheads), which exposed the ZPS. Papillose outgrowths from the zoosporangium were evident and the apical portion of the nipple-like protuberance was ruptured, forming an escape pore (E) through which zoospores would eventually be released. This escape pore was occluded with a material that did not protrude from the pore, but could be seen (arrow) within the pore (Fig. 40). Later in the abscission process the ZPS was split (Fig. 41), and frequently the zoosporangium appeared to be hinged on its pedicel (Fig. 42). This hinged arrangement of zoosporangium on pedicel was created by an incomplete separation of the ZPS and continued tearing of the outer septal wall.

Denuded pedicels (e.g. Fig. 43) contained a central hatch-like protuberance (H) which emerged from within the split ZPS. The hatch-like appearance (Fig. 43) indicates that as the protuberance emerged, it broke through the surface layer ZPS wall material. A similar central hatch-like (H) protuberance occurred in the abscised zoosporangium (Fig. 44), and frequently the entire ZPS bulged outward.

Zoosporogenesis and Zoospores - Fine Structure

Zoosporogenesis paralleled the external events of zoosporangial development (Fig. 45-51). Zoosporangia slightly more mature than the rugulose stage (Fig. 45, cf. Fig. 36) contained numerous discoidal chloroplasts (C) with starch grains (S), haematochrome inclusions (h), elongated mitochondria (M), and a large (ca. 3 μ m in diameter) nucleus (N) with a single nucleolus (Nu). The zoosporangial wall contained densely staining sporopollenin which was more prominent in the outer portion of the sporangial wall, thereby separating the wall into a densely stained outer zone (OZ) and a lightly stained inner zone (IZ). In larger, more mature zoosporangia (Fig. 46), densely staining smaller (ca. 1.5 μ m in diameter) nuclei (N) were present. Starch grains (S) were larger than those seen in less mature zoosporangia; haematochrome inclusions (h) were more numerous, and flagella (at black arrows) were visible. An escape pore occluded with material (PM) was well developed. Papillae (hollow arrowheads) composed of densely staining zoosporangial wall material were present. Cytoplasmic cleavage (Fig. 47) was evidenced by the presence of elongate (as seen in section) vesicles (arrows) which at later stages (Fig. 48-50) fused and clearly demarcated individual zoospores. The zoospores contained discoidal chloroplasts with starch grains, haematochrome, and elongate mitochondria. Eye-spots were never observed. As seen in thin-section, a mature zoosporangium (Fig. 51) contained several zoospores (six in Fig. 51). Whereas, the unsplit ZPS (Fig. 52) of less mature zoosporangia contained an unbroken outer ring (OR) and an inner ring (IR)

of septal wall material, mature zoosporangia (Fig. 53) contained an outer ring which was torn open (arrows) and an intact ZPS inner area. Plasmodesmata (ca. 40-50 nm in diameter) shown in the inner ZPS area of a mature zoosporangium (Fig. 53) were present in the septa from the earliest stages in zoosporogenesis.

Flagellar Apparatus of Zoospores

All negatively stained zoospores appeared to be biflagellate (Fig. 54) with the flagella inserted into an anterior apical papilla (AP). However, scanning electron microscopy frequently showed that apparent biflagellate cells were actually quadriflagellate (Fig. 55-61). Single appearing flagella frequently consisted of two flagella (Fig. 55) that were tightly appressed (arrows). Further, it was frequently observed that the flagella could be slightly separated (Fig. 56 and 57) or completely separated (Fig. 58). Different aspects of flagellar insertion into the prominent apical papilla are shown in Figures 59 through 61.

Each zoospore (two of which are shown in serial sections, Fig. 62 and 63) had two upper (U) and two lower (L) basal bodies which were parallel and overlapping. The proximal portions of basal bodies were covered with an electron dense material that formed a flagellar cap. One such flagellar cap is shown in Figure 63 (arrowheads). Part of the nucleus (N) projected between the lower pair of basal bodies (Fig. 62 and 63, 65), and an electron dense line covered the upper basal bodies, forming a distal band (DB) which was frequently seen to extend over the entire apical papilla (Fig. 62 and 63). The upper basal bodies

(Fig. 64, arrows) were inserted into the apical papilla from opposite directions, as were the lower basal bodies.

The multilayered structures (portions of each shown between arrowheads), were associated with each of the two upper basal bodies (Fig. 65). Figure 66 shows one of these multilayered structures (between arrowheads) abutted to the proximal area of the upper basal body (B). Spline microtubules (SM), part of the dorsal surface of the multilayered structure, extended posteriorly beneath the plasmalemma (PL) of the motile cell. In addition, other spline microtubules (SM) were located beneath the plasmalemma on the opposite side of the cell. Although out of the plane of section, these spline microtubules were part of another multilayered structure which connected with the other upper basal body. Schematic diagrams of a zoospore showing the proposed relative positions of basal bodies, flagella, multilayered structures, and other cellular organelles are shown in Figure 67.

As seen in cross-section (Fig. 68-70), flagella possessed a typical "9+2" microtubule arrangement. Often a row of spline microtubules (SM) was present beneath the plasmalemma (Fig. 68). Lateral flagellar extensions or keels (Fig. 69 and 70) were frequently observed, and occasionally, microtubule-like structures (arrows) were present in these keels.

Gametangia and Gametogenesis

Gametangia (Fig. 71) were formed at all positions on the dorsal surface of the algal thallus and were most abundant in thalli collected during the summer months. Escape pores, through which gametes would

eventually be released, formed on the upper surfaces of intercalary gametangia (Fig. 72) or on gametangia located at the margins of thalli (Fig. 73). The escape pores (Fig. 73) were frequently occluded with material (arrow) that did not protrude from the pores.

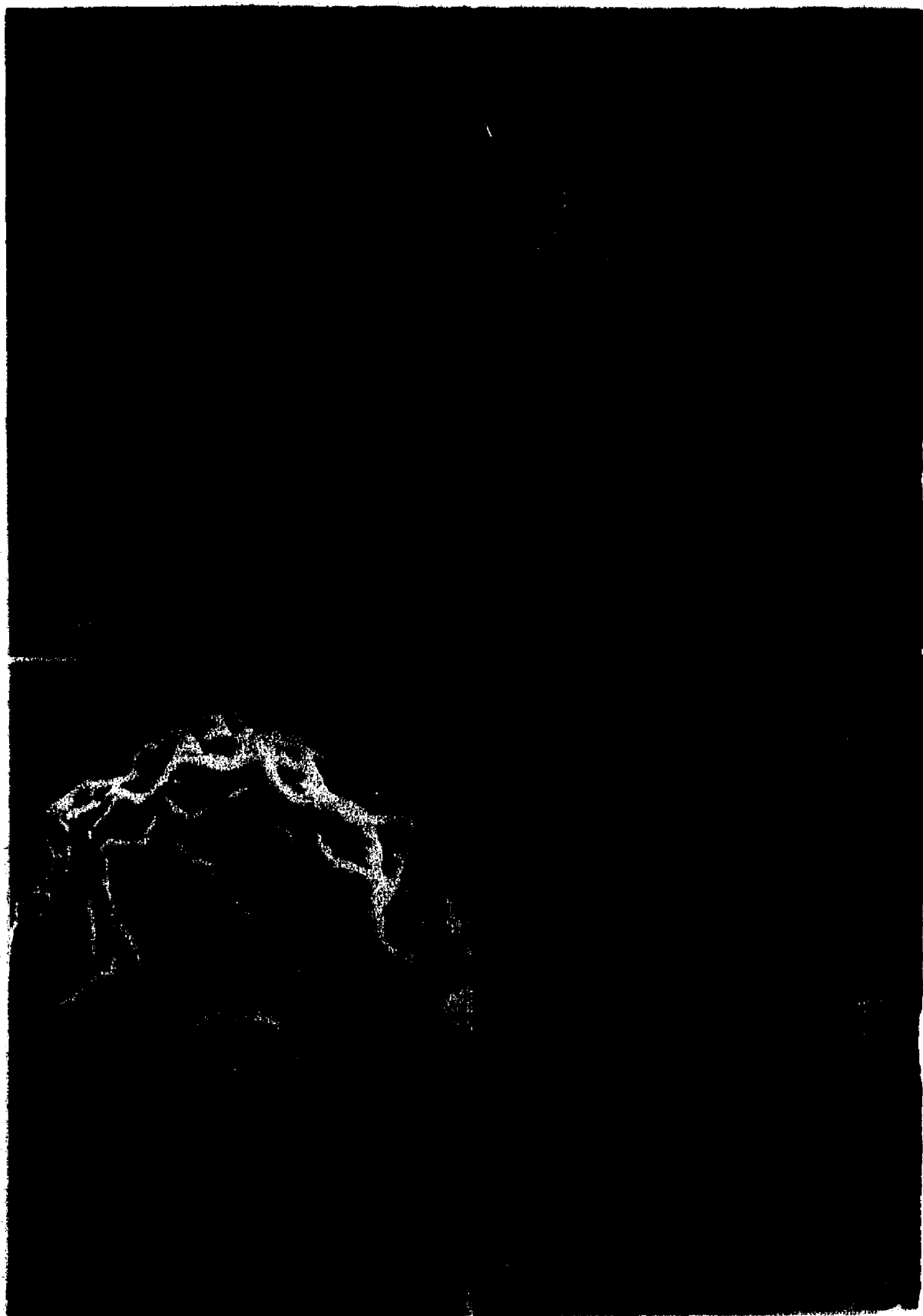
Early in gametogenesis, the gametangia contained nuclei (N) that did not stain densely (Fig. 74). Further, flagella were never observed at this stage of gametic development. At a later stage (Fig. 75), cytoplasmic cleavage was initiated, nuclei appeared more compact and densely stained, flagella (arrows) were apparent, and pores with occluding pore material (PM) were present. In more mature gametangia further cytoplasmic cleavage was evident (Fig. 76), and separate uninucleate gametes were present in very mature gametangia (Fig. 77). Each gamete contained haematochrome inclusions, mitochondria and discoidal chloroplasts. The plastids contained starch grains, but lacked pyrenoids. Eye-spots were never observed. The gametangial wall that surrounded the occluded escape pore in less mature cells (Fig. 78, arrow) was dispersed in more mature gametangia (Fig. 79). The occluding pore material (PM) stained densely at later stages (e.g. Fig. 80), and was eventually gone from the pore of empty gametangia (e.g. Fig. 81).

Flagellar Apparatus of Gametes

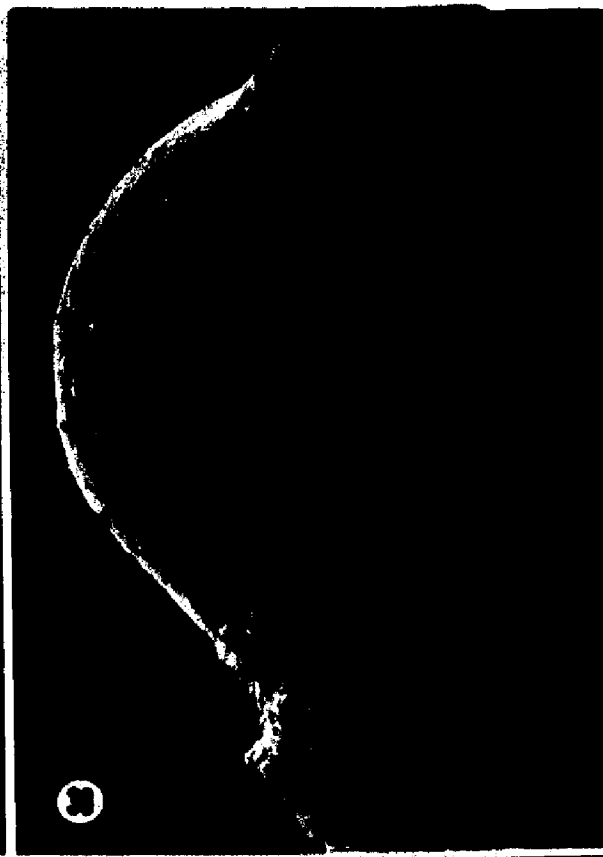
Gametes possessed two keeled flagella (F) that inserted into an anterior apical papilla from opposite sides (Fig. 82). In addition, a nucleus (N) was located immediately beneath the parallel, overlapping flagellar bases (Fig. 82); and spline microtubules (SM), located next

to the plasmalemma (PL), terminated in a multilayered structure (a portion of which is between arrowheads) which was in turn connected to a basal body (B). Frequently, spline microtubules (SM) which were part of a multilayered structure (between arrowheads) were observed on the opposite side of a gamete (Fig. 83; cf. Fig. 82 and 84). In addition, the electron dense lowest stratum of the multilayered structure was adjacent to the nucleus. Occasionally, two separate sets of spline microtubules and/or portions of two multilayered structures (arrows) were observed in the same motile cell (Fig. 85-87). Proximally, the basal bodies (B) possessed flagellar caps (arrows) composed of electron dense material (Fig. 88 and 89) and a distal band (DB) was frequently seen between the plasmalemma and basal bodies of the motile cell (Fig. 90 and 91). Schematic overhead and side views of a gamete (Fig. 92 and 93) show the proposed relative positions of basal bodies, multilayered structures, and other organelles.

- Fig. 34. Pedicels bearing terminal zoosporangia on dorsal surface of Phycopeltis epiphyton. Zoosporangia are at different developmental stages. Scanning electron microscope (SEM) micrograph. X 2,000.
- Fig. 35. Early developmental stage of zoosporangium (Z) at apical end of pedicel (P) in P. epiphyton. Note rugulose wall of immature zoosporangium, septal ridge (arrows), and exposed portion of zoosporangium-pedicel septum (between arrowheads). SEM micrograph. X 13,000.
- Fig. 36. Later developmental stage of P. epiphyton zoosporangium. SEM micrograph. X 9,200.



- Fig. 37. Later developmental stage of P. epiphyton zoosporangium. Zoosporangium is expanded and small papillose outgrowth (arrows) are present on pedicel. SEM micrograph. X 5,400.
- Fig. 38. Nearly mature P. epiphyton zoosporangium with terminal nipple-like protuberance (arrow). SEM micrograph. X 5,250.
- Fig. 39. Zoosporangial abscission in P. epiphyton. Outer septal (lateral) wall is torn exposing zoosporangium-pedicel septum (between arrowheads). Well developed escape pore (E) and papillose outgrowths are present. SEM micrograph. X 6,000.
- Fig. 40. Material (arrow) occluding zoosporangial escape pore of P. epiphyton. SEM micrograph. X 8,800.



- Fig. 41. Torn outer septal (lateral) wall around zoosporangium-pedicle septum in P. epiphyton. SEM micrograph. X 13,000.
- Fig. 42. Hinged aspect of partially abscised P. epiphyton zoosporangium. SEM micrograph. X 5,400.
- Fig. 43. Hatch-like central protuberance (H) on a denuded pedicle of P. epiphyton. SEM micrograph. X 9,750.
- Fig. 44. Released zoosporangium of P. epiphyton with hatch-like central protuberance (H) similar to denuded pedicle (cf. Fig. 43). SEM micrograph. X 7,500.

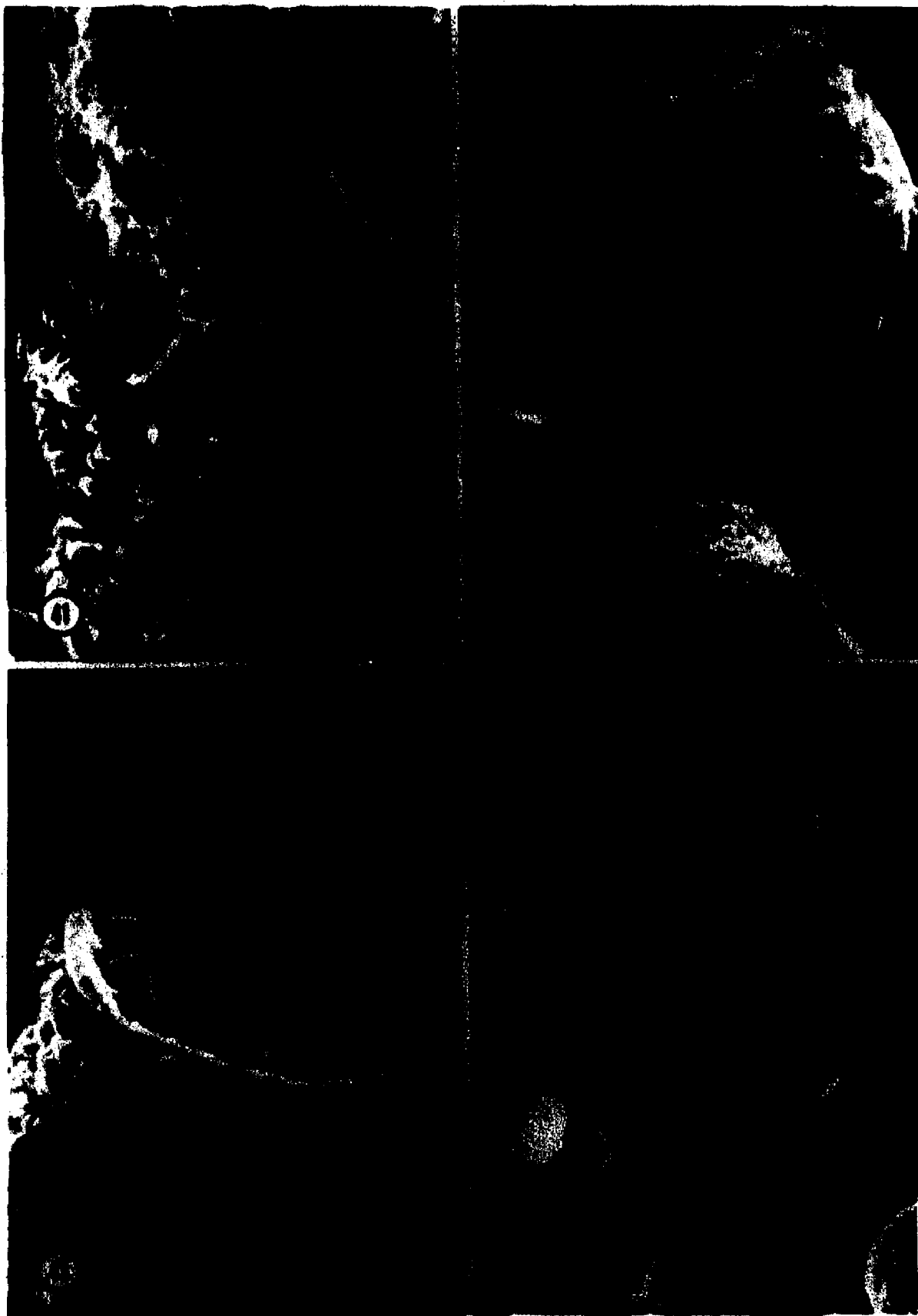


Fig. 45. Immature zoosporangium of P. epiphyton. Densely staining outer zone (OZ) and lighter staining inner zone (IZ) present in wall. Note presence of chloroplasts (C), starch grains (S), haematochrome (h), mitochondria (M), and large nucleus (N) with single nucleolus (Nu). Transmission electron microscope (TEM) micrograph. X 31,000.

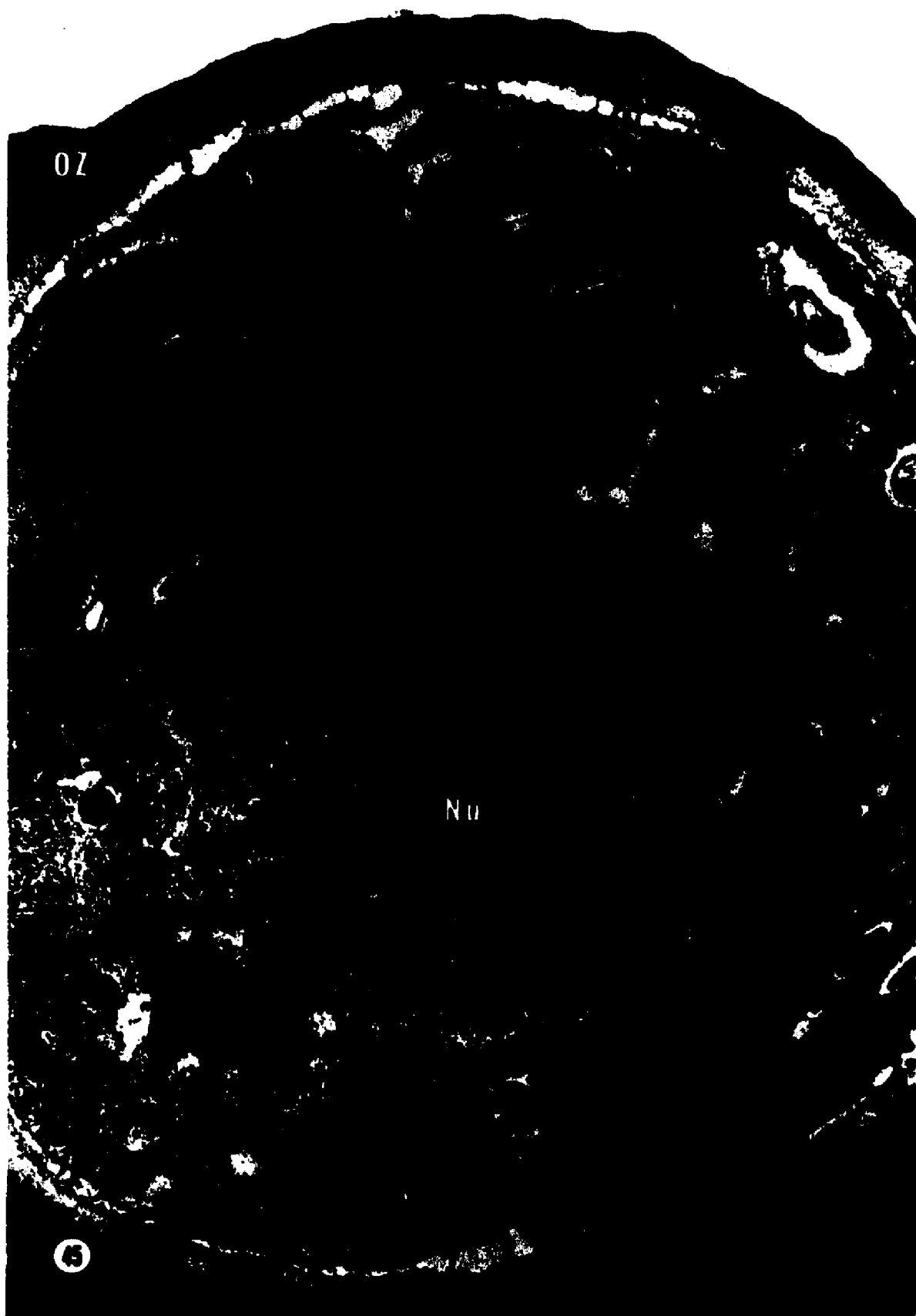
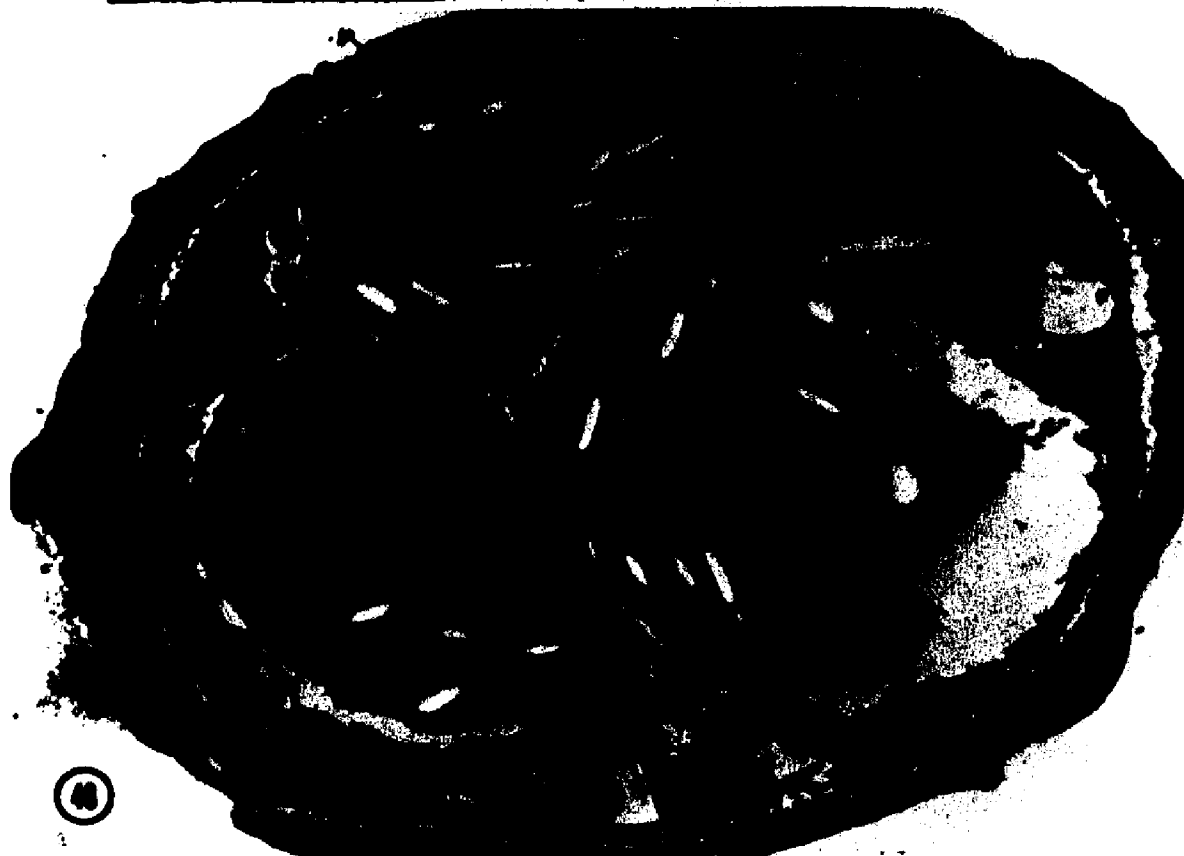


Fig. 46. Pre-cytokinetic stage in P. epiphyton zoosporogenesis. Note presence of flagella (black arrows), small nuclei (N), occluding pore material (PM), and abundance of haematochrome (h) and starch grains (S). Papillose outgrowths (hollow arrowheads) on zoosporangium wall formed by densely staining material are also evident. TEM micrograph. X 14,000.



Fig. 47. Incipient cytokinesis in multinuclear P. epiphyton zoosporangium evidenced by formation of elongate vesicles (arrows). TEM micrograph. X 9,450.

Fig. 48. Later stage in cytokinesis in P. epiphyton zoosporangium. Elongated vesicles have fused and partial segregation of cytoplasm into discrete masses is apparent. TEM micrograph. X 10,000.



- Fig. 49. Nearly mature P. epiphyton zoospores. Note prominent flagella and increased disorganization of general cytoplasm (cf. Fig. 48). TEM micrograph. X 6,600.
- Fig. 50. Mature zoosporangium containing clearly visible zoospores (e.g. ZP). Some zoosporangial cytoplasm remains unincorporated. TEM micrograph. X 10,350.

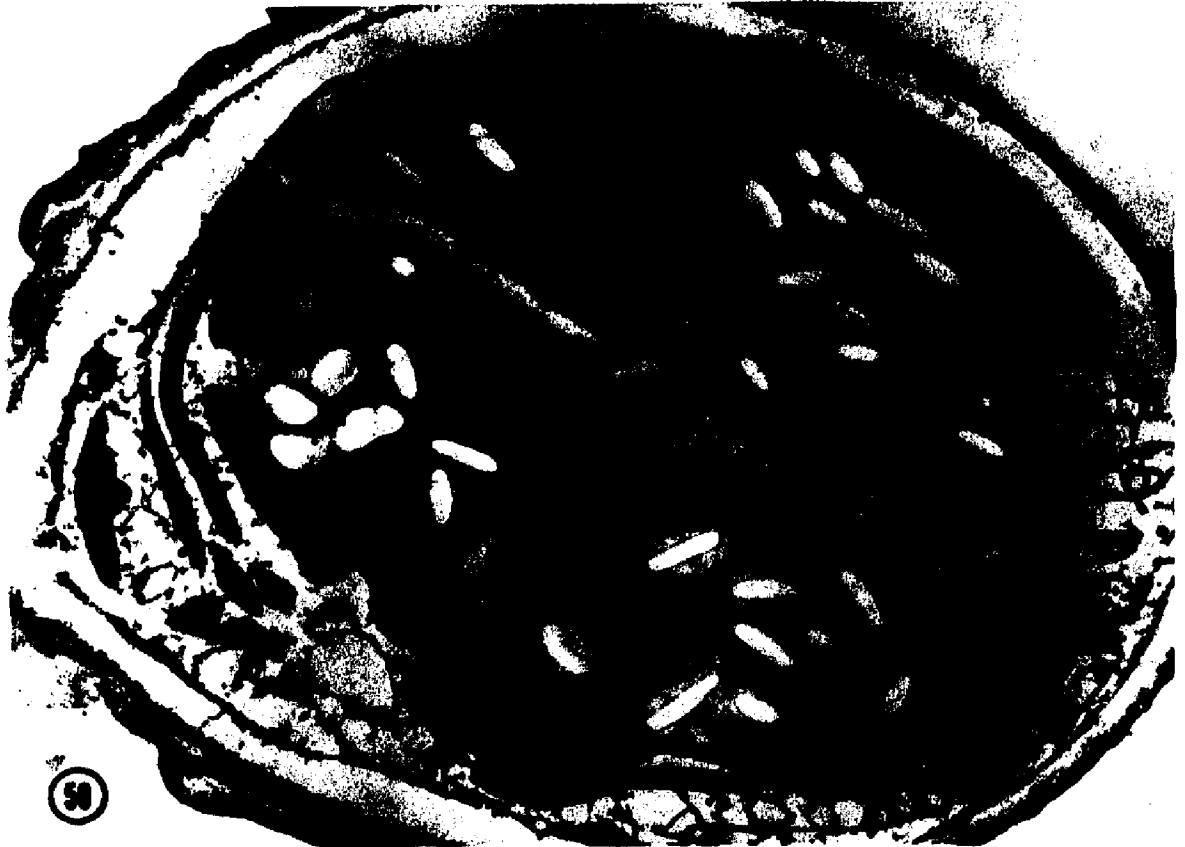


Fig. 51. Mature zoosporangium of P. epiphyton containing at least six zoospores (e.g. ZP). Note torn outer ring (arrows) of zoosporangium-pedicel septum (cf. Fig. 52 and 53) and escape pore material (PM). TEM micrograph. X 14,500.



- Fig. 52. Immature zoosporangium of P. epiphyton wherein both outer ring (OR) and inner ring (IR) composed of zoosporangium-pedicel septum wall material are unbroken. TEM micrograph. X 18,000.
- Fig. 53. Mature zoosporangium of P. epiphyton wherein outer ring (arrows) has broken; inner area of zoosporangium-pedicel septum is intact. TEM micrograph. X 18,200.



- Fig. 54. Negatively stained apparently biflagellate zoospore of P. epiphyton. Note "two" flagella insert into anterior apical papilla (AP). TEM micrograph. X 20,800.
- Fig. 55. Zoospore of P. epiphyton in which paired flagella (arrows) are tightly appressed. SEM micrograph. X 22,500.
- Fig. 56. Zoospore of P. epiphyton in which flagella are slightly separated. SEM micrograph. X 9,450.
- Fig. 57. Detailed view of slightly separated paired flagella in zoospore of P. epiphyton. SEM micrograph. X 42,000.



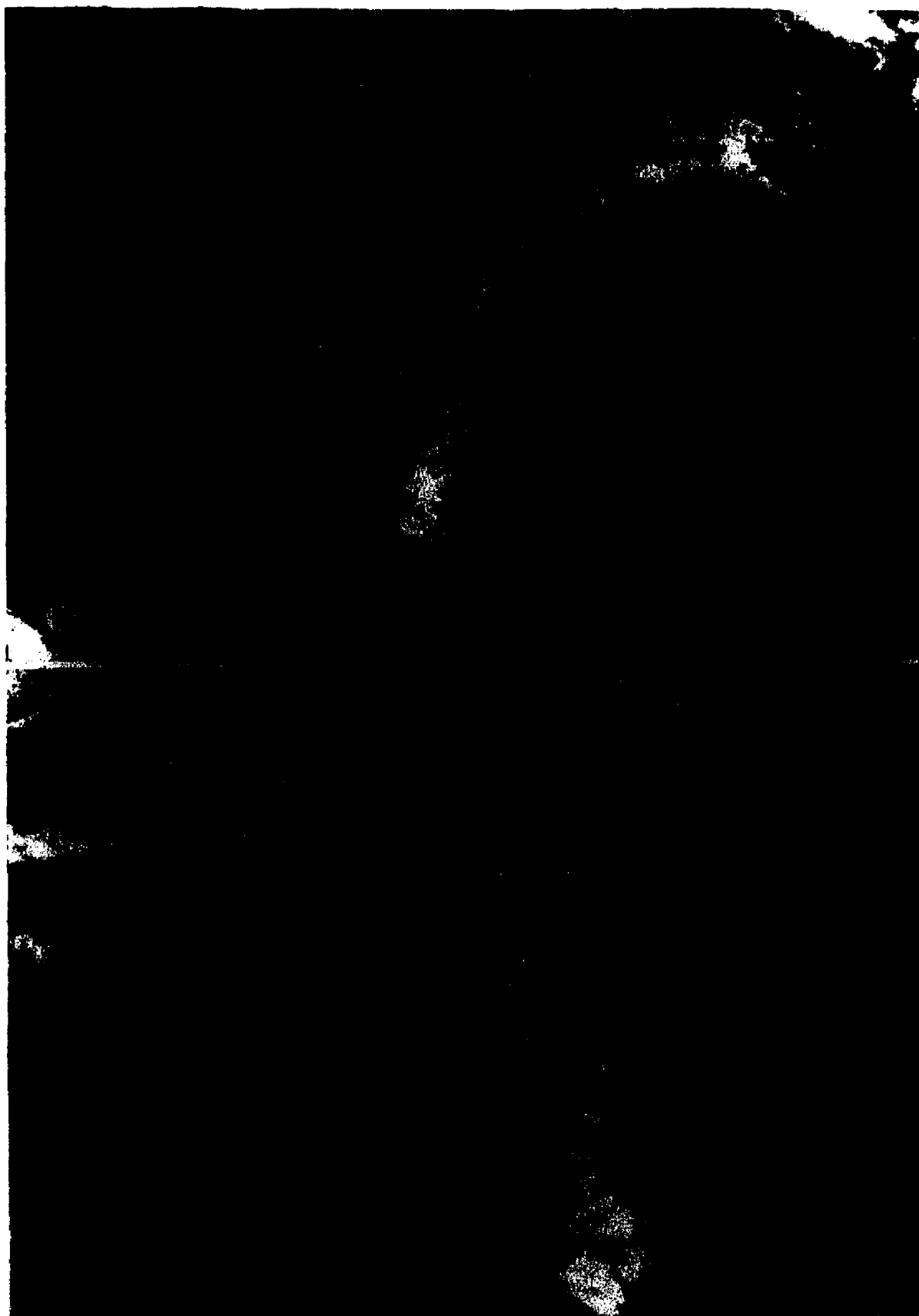
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- Fig. 58. Completely separated paired flagella of P. epiphyton zoospore. SEM micrograph. X 4,900.
- Fig. 59. Four flagella inserted into apical papilla (AP) of P. epiphyton zoospore. SEM micrograph. X 28,000.
- Fig. 60. Flagella insertion into apical papilla (AP) of P. epiphyton zoospore in anterior view. SEM micrograph. X 28,000.
- Fig. 61. Flagella insertion into apical papilla (AP) in P. epiphyton zoospore in side view. SEM micrograph X 35,000.



- Fig. 62. Two P. epiphyton zoospores each containing two upper (U) and two lower (L) basal bodies. Note nucleus (N) projecting between lower pair of basal bodies and electron dense distal band (DB) covering upper pair of basal bodies. Transverse section. TEM micrograph. X 97,000.
- Fig. 63. Serial section of zoospores in Figure 61. Note distal band (DB) and electron dense material forming flagellar cap (arrowheads). Transverse section. TEM micrograph. X 97,000.



- Fig. 64. Upper basal bodies (arrows) of P. epiphyton zoospore inserted into apical papilla. Longitudinal section. TEM micrograph. X 81,000.
- Fig. 65. Portions of two multilayered structures (between arrowheads) associated with two upper basal bodies (U) in P. epiphyton zoospore. Oblique section. TEM micrograph. X 81,200.
- Fig. 66. One multilayered structure (between arrowheads) abutted on proximal portion of upper basal body (U) in P. epiphyton zoospore. Note spline microtubules (SM) immediately beneath plasmalemma (PL). Longitudinal section. TEM micrograph. X 61,500.

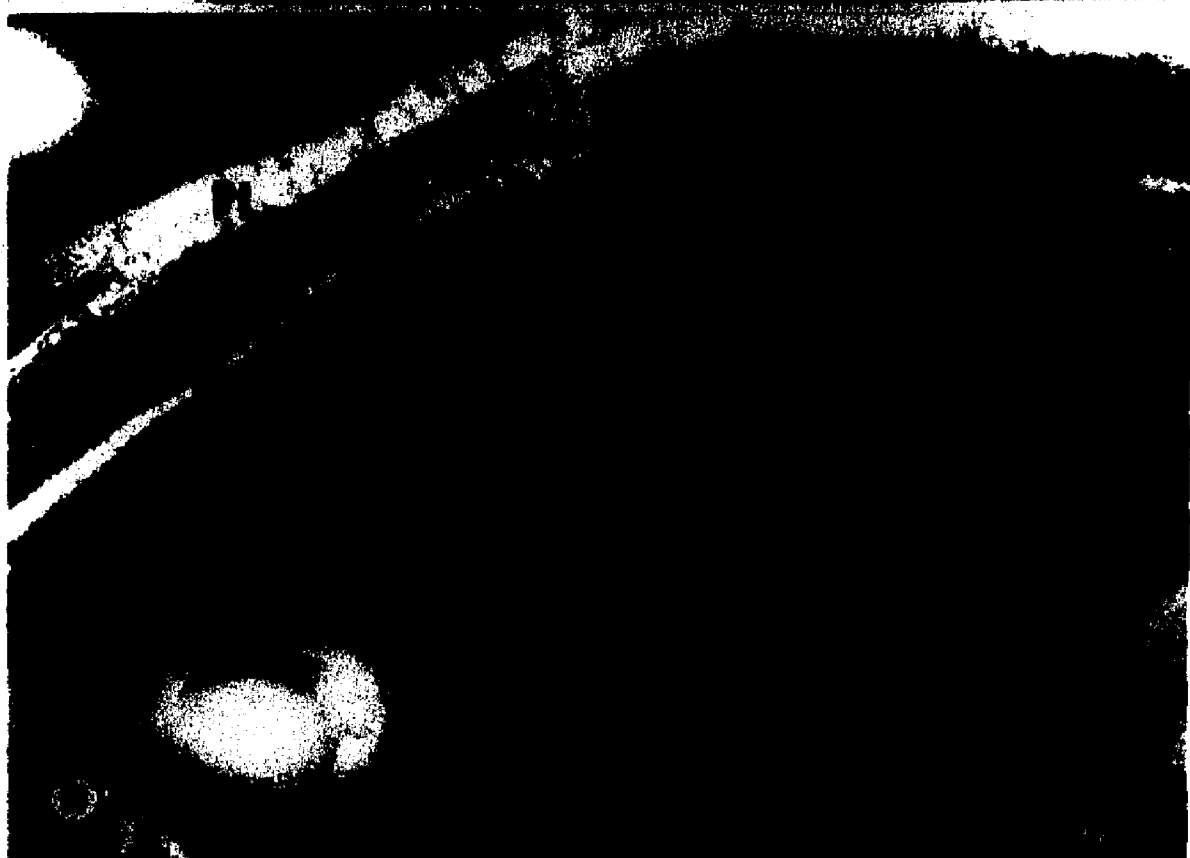
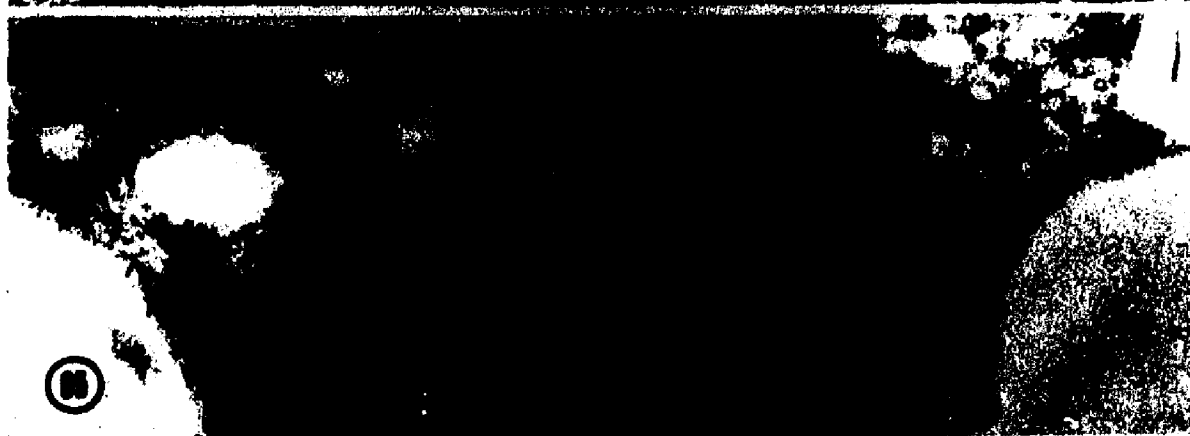
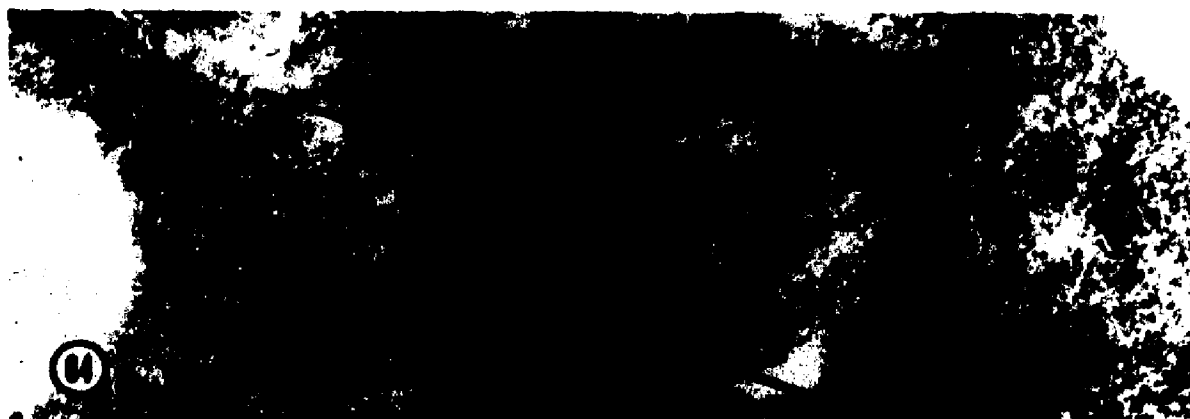
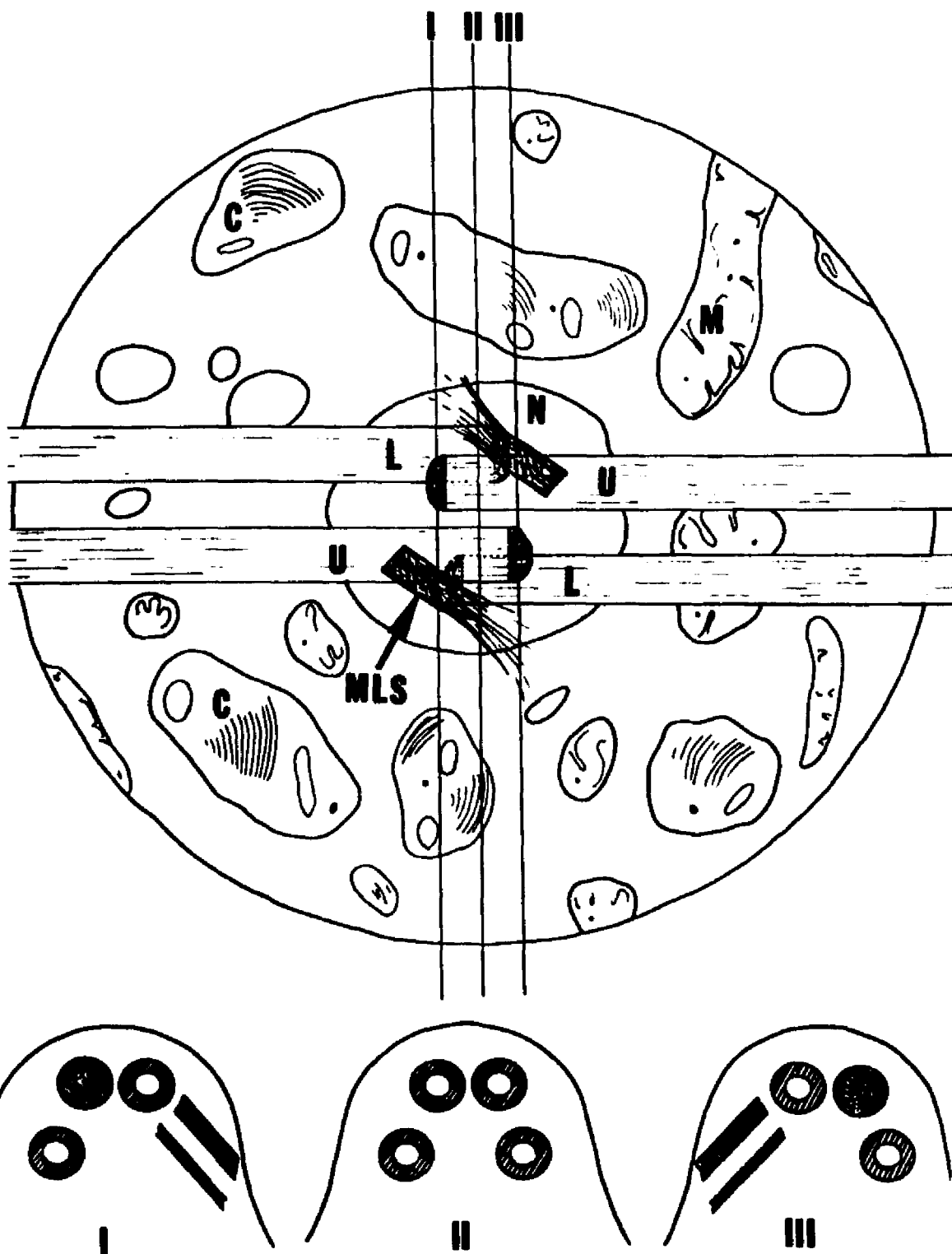


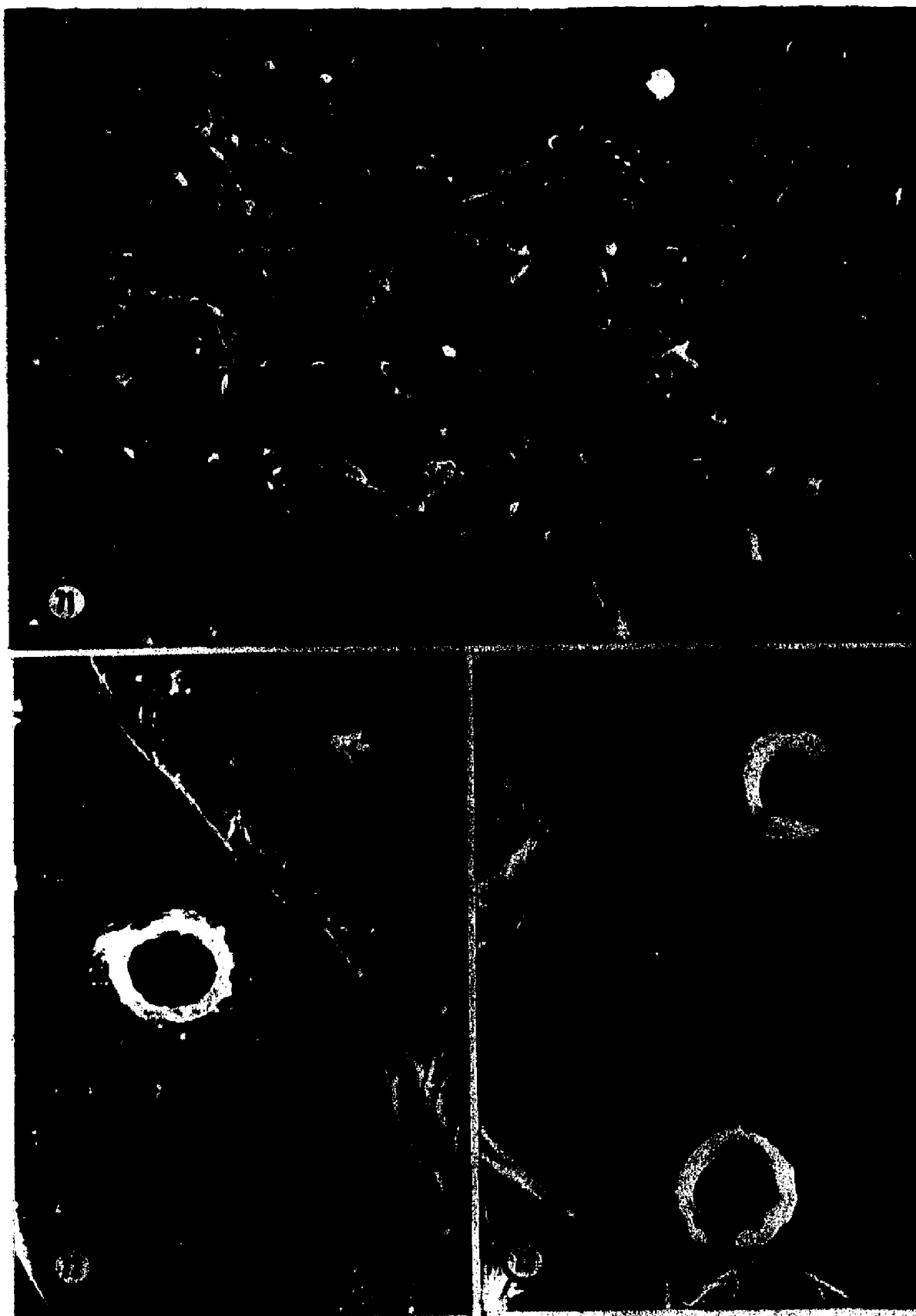
Fig. 67. Schematic overhead view of proposed flagellar apparatus in P. epiphyton quadriflagellate zoospore. All basal bodies are parallel and overlapping. Note upper basal bodies (U) are more centrally located than lower basal bodies (L); also flagella arising from one lower basal body and one non-adjacent upper basal body emerge from either side of the apical papilla. Densely staining flagellar caps are present at the proximal end of each basal body. Arrangement of basal bodies and multilayered structures (MLS) as seen in three different planes of transverse section (I, II, III) through apical papilla are also shown (cf. Fig. 62 and 63). C, chloroplast; M, mitochondrion; N, nucleus.



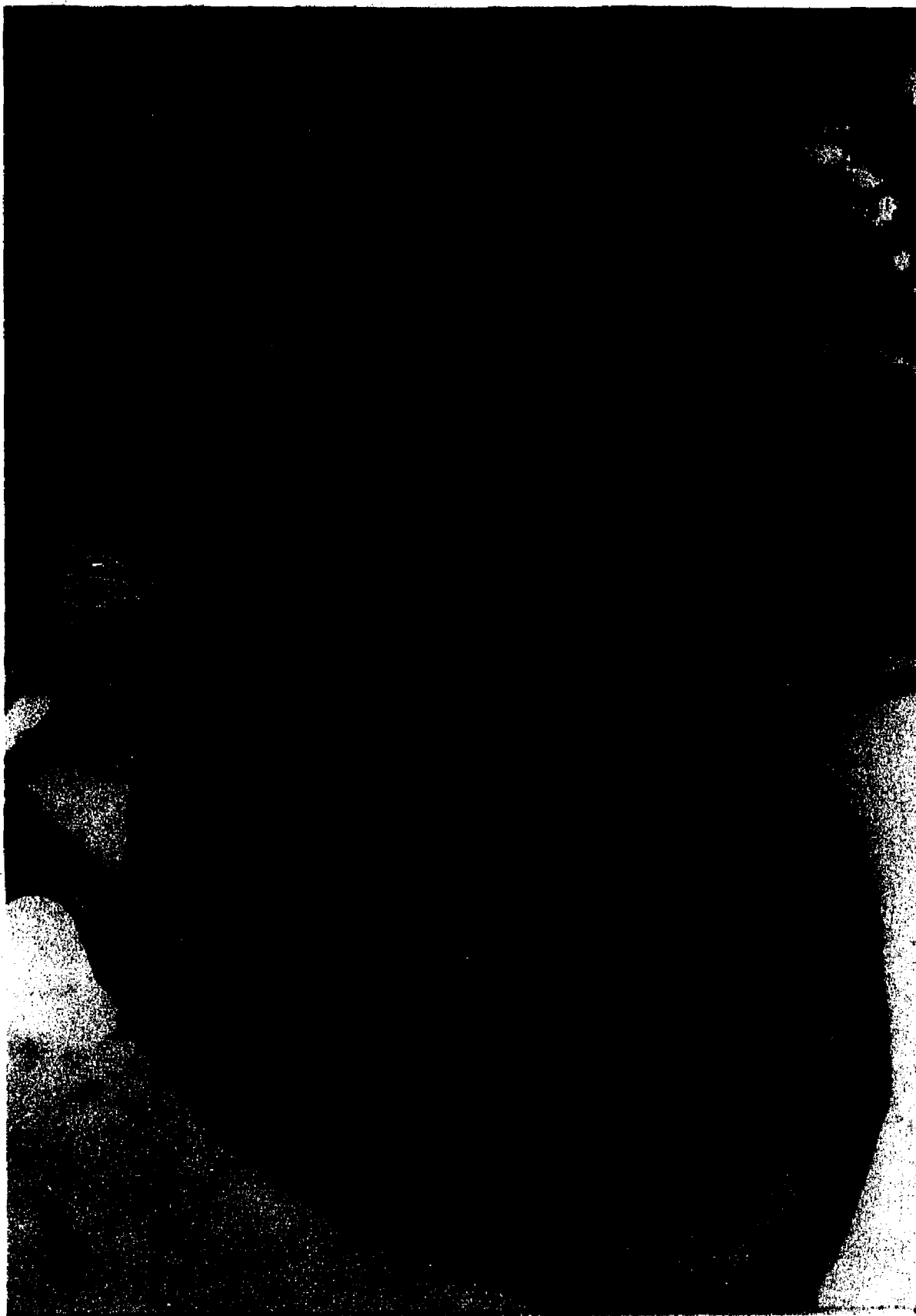
- Fig. 68. Typical "9+2" microtubule arrangement occurs in the flagella of zoospores in P. epiphyton. Note the spline microtubules (SM) immediately beneath the plasmalemma (PL). Transverse section. TEM micrograph. CW, cell wall. X 132,000.
- Fig. 69. Lateral extensions (keels) present in flagella of P. epiphyton zoospores. Microtubule-like structures (arrows) are occasionally observed. Transverse section. TEM micrograph. X 108,000.
- Fig. 70. Two closely appressed flagella of P. epiphyton zoospores. Note flattened appearance caused by elongated keels. Also, microtubule-like structures (arrows) are shown. TEM micrograph. X 135,700.



- Fig. 71. Gametangia (e.g. arrows) on dorsal surface of P. epiphyton. SEM micrograph. X 540.
- Fig. 72. Intercalary gametangium of P. epiphyton. SEM micrograph. X 5,400.
- Fig. 73. Marginal gametangia of P. epiphyton. Note escape pore occluded with material (arrow). micrograph. X 5,100.



- Fig. 74. Early stage of gametogenesis in P. epiphyton. Three lightly staining nuclei (e.g. N) present. Paradermal section. TEM micrograph. X 7,500.
- Fig. 75. Later stage of gametogenesis in P. epiphyton. Some cytoplasmic cleavage evident. Note occluding pore material (PM), flagella (arrows), and densely staining nuclei (three of which are visible). Paradermal section. TEM micrograph. X 16,000.



- Fig. 76. Cleaved cytoplasm at later stage of gametogenesis in P. epiphyton. Cytoplasmic disorganization is pronounced and uninucleate gametes are discernable. Paradermal section. TEM micrograph. X 11,000.
- Fig. 77. Pre-release stage of gametogenesis in P. epiphyton. Note seven uninucleate gametes and remnants of unincorporated cytoplasm. Paradermal section. TEM micrograph. X 7,200.



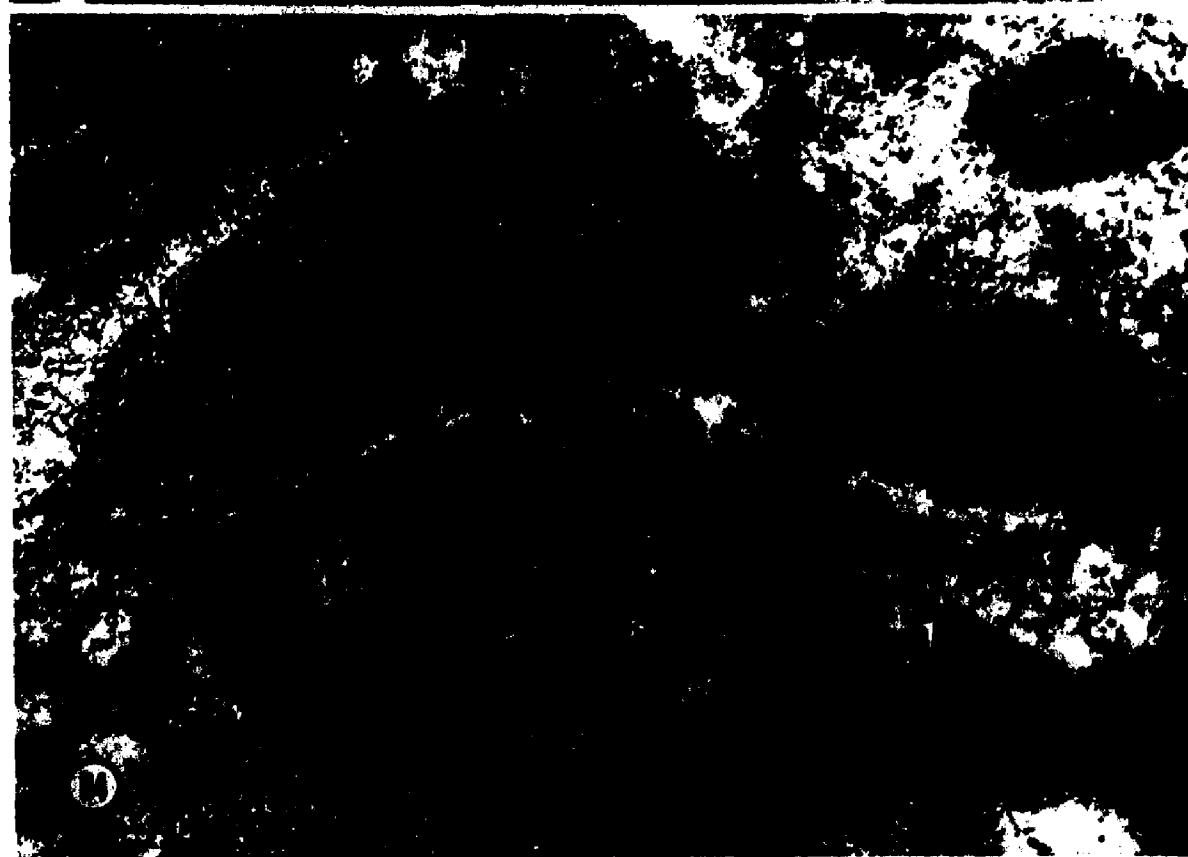
- Fig. 78. Intact gametangial wall (arrow) in P. epiphyton surrounds occluding pore material. Paradermal section. TEM micrograph. X 9,000.
- Fig. 79. Early stage of gametangium escape pore formation in P. epiphyton. Wall surrounding occluding pore material is thin (arrows). Paradermal section. TEM micrograph. X 18,200.
- Fig. 80. Later stage of escape pore formation in P. epiphyton. Wall surrounding occluding pore material (PM) is torn (arrows). Note densely staining pore material (PM). Paradermal section. TEM micrograph. X 33,600.
- Fig. 81. Empty gametangium of P. epiphyton. Pore material is gone and gametes have been released. Paradermal section. TEM micrograph. X 7,500.



Fig. 82. Biflagellate gamete of *P. epiphyton*. Note spline microtubules (SM) beneath the plasmalemma (PL) terminate in a multilayered structure (between arrowheads) connected to a basal body (B). Longitudinal section. TEM micrograph. F, flagella; N, nucleus. X 115,000.



- Fig. 83. Gamete of P. epiphyton containing spline microtubules (SM) which terminate in a multilayered structure (between arrowheads) connected to a basal body. Multilayered structure is adjacent to the nucleus (N). Longitudinal section. TEM micrograph. F, flagella. X 55,000.
- Fig. 84. Two overlapped basal bodies in P. epiphyton gametes. Spline microtubules (SM) terminate in a multilayered structure (a portion of which is visible, arrow). Oblique section. TEM micrograph. X 82,800.



- Fig. 85. One multilayered structure (between arrowheads), and two sets of spline microtubules (arrows) visible in P. epiphyton gamete. Longitudinal section. TEM micrograph. X 74,800.
- Fig. 86. Portions of two multilayered structures (arrows) visible in P. epiphyton gamete. Longitudinal section. TEM micrograph. X 64,000.
- Fig. 87. Portions of two multilayered structures (arrows) adjacent to basal bodies visible in P. epiphyton gamete. Oblique section. TEM micrograph. X 67,200.



- Fig. 88. Proximal regions of two basal bodies (B) with electron dense flagellar cap (arrow) in gametes of P. epiphyton. Transverse section. TEM micrograph. X 76,800.
- Fig. 89. Overlapped, adjacent basal bodies in P. epiphyton gamete. Note one basal body was sectioned through its flagellar cap (arrow), the other at a plane distal to its flagellar cap. Transverse section. TEM micrograph. X 85,000.
- Fig. 90. Two portions of distal band (DB) above two basal bodies in gamete of P. epiphyton. Transverse section. TEM micrograph. X 57,200.
- Fig. 91. Complete distal band (DB) above two basal bodies in gamete of P. epiphyton. Transverse section. TEM micrograph. X 52,200.

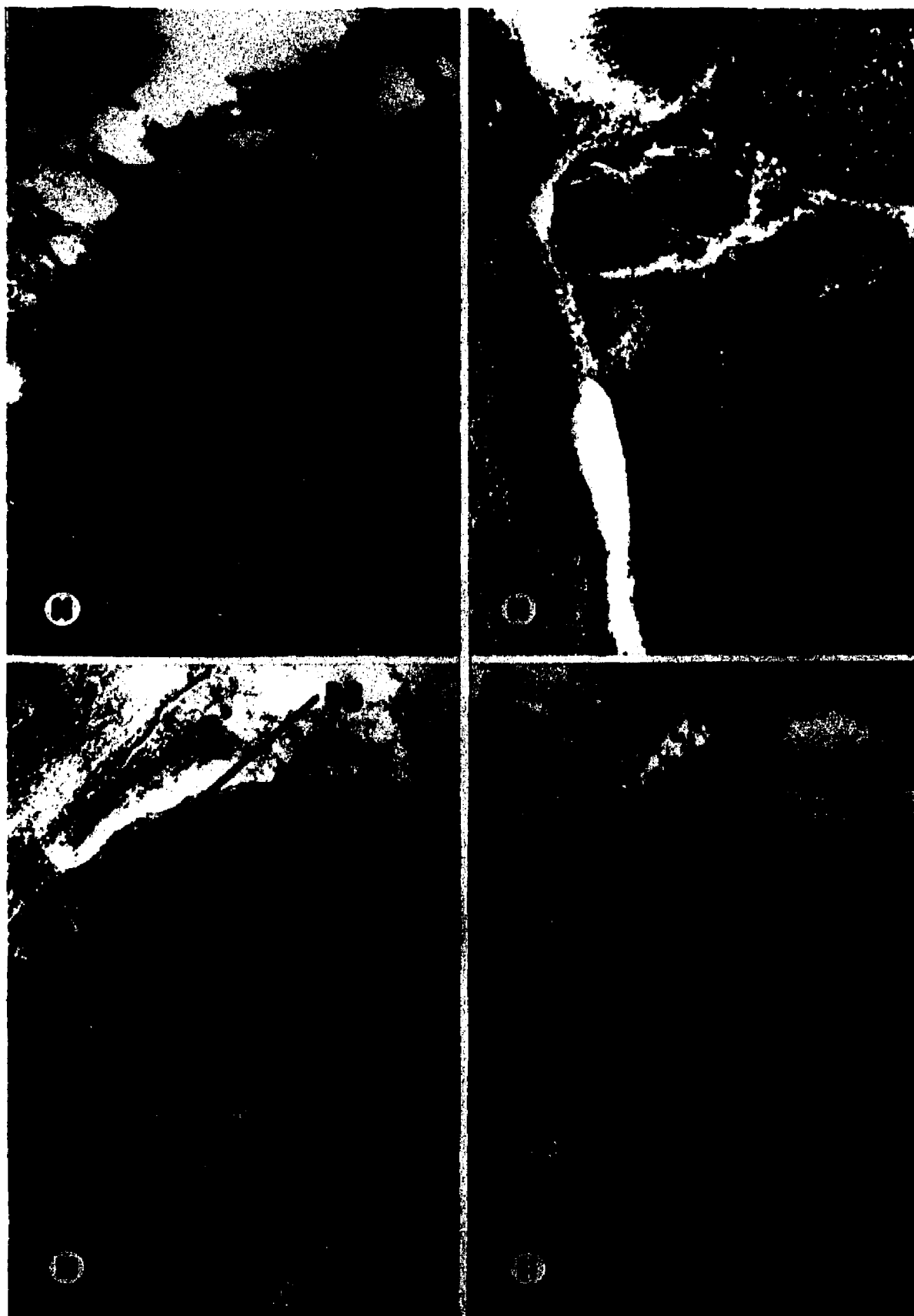
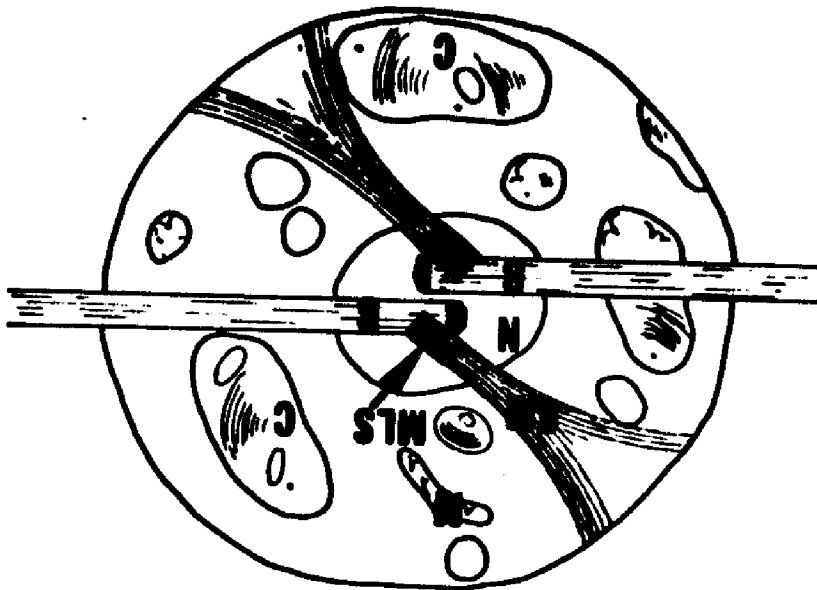


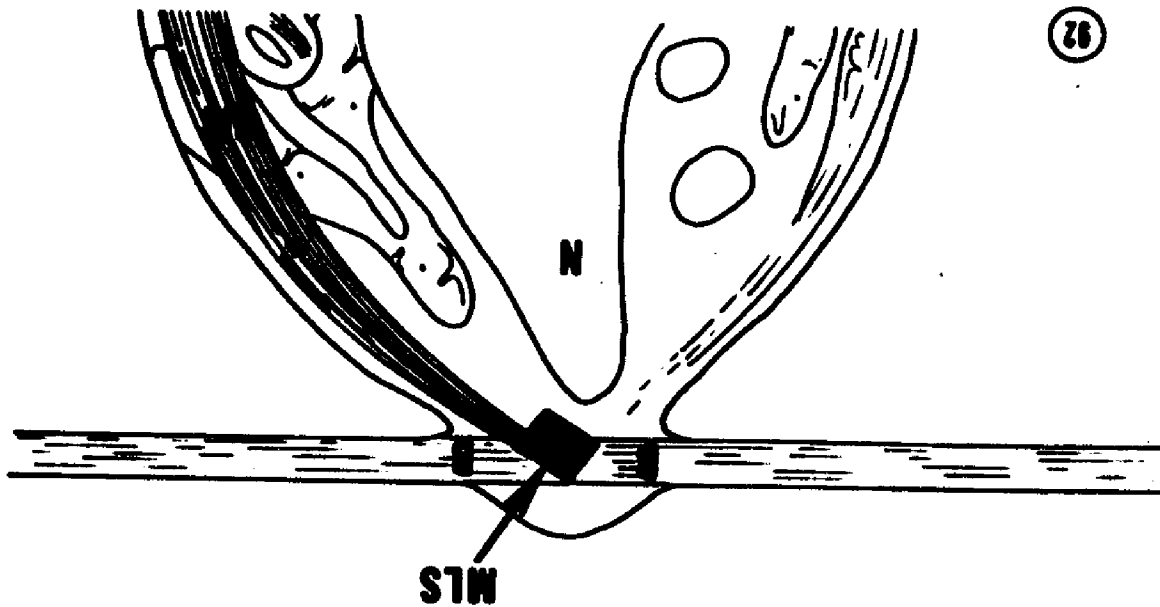
Fig. 92. Schematic side view of proposed flagellar apparatus in P. epiphyton biflagellate gamete. Basal bodies (B) are parallel, overlapped, and in same horizontal plane. Only one multilayered structure (MLS) is visible in this view. Note close association between flagellar apparatus and nucleus (N) and that spline microtubules (SM) are immediately beneath plasmalemma.

Fig. 93. Schematic overhead view of proposed flagellar apparatus in P. epiphyton biflagellate gamete. The two basal bodies (B) are parallel and overlapping and two multilayered structures (MLS) are visible in this view. Densely staining flagellar caps are present at the proximal end of each basal body. Note bifurcation of spline microtubules (SM). C, chloroplast; M, mitochondrion; N, nucleus.

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Discussion and Conclusions

Zoosporangia

In general the scanning electron microscope observations in this study parallel those reported for zoosporangial development and subsequent abscission in Cephaleuros virescens (Chapman, 1976). The observed seasonal proliferation of zoosporangia in April through September is undoubtedly the result of favorable environmental factors (e.g. light, moisture, temperature). The rugulose appearing wall at the distal end of a pedicel may be typical of apical growth and most certainly indicates an early stage of zoosporangial initiation. The disappearance of these rugulae during later stages of zoosporangial maturation (also observed in C. virescens) is presumably the result of zoosporangial expansion. The observation that similar rugulae are present in immature zoosporangia of C. virescens prepared for transmission electron microscopy (Dr. R. L. Chapman, personal communication) indicates that the rugulae probably are not artifacts.

The septal ridge that forms beneath the rugulate pedicel apical area indicates the position of the zoosporangial-pedicel septum (ZPS). Because no incomplete ZPS was observed, it is likely that the formation of this partition is quite rapid once initiated. Therefore, it seems probable that when the septal ridge is observed, the ZPS has been completely formed. The plasmodesmata in the central area of the ZPS provide protoplasmic continuity; hence the movement of

water and perhaps photosynthate may continue into the developing zoosporangium.

The densely staining material that divides both pedicel and zoosporangium walls into an outer densely staining zone and an inner less densely staining zone is a sporopollenin (see preceding chapter and Good and Chapman, 1978a), and the papillae that eventually form on both pedicel and zoosporangial walls are aggregations of this substance. The primary function of this substance may be to protect zoospores contained within a zoosporangium from desiccation and/or parasitism (e.g. by fungi). In addition, the papillae of zoosporangia might in some way enhance the adhesion of wind- or water-borne zoosporangia to the leaf surfaces of new hosts.

It is clear that the lightly stained enlarged nucleus of an immature zoosporangium (Fig. 47) signals impending karyokinesis. A similar nucleus at an equivalent stage of zoosporangial development also occurs in C. virescens (Dr. R. L. Chapman, unpublished results). Large lightly stained nuclei such as these undoubtedly give rise to the small densely stained nuclei of zoospores. Similar densely staining nuclei have been observed in the motile cells of Cephaleuros and Trentepohlia (Graham and McBride, 1975; Chapman and Good, 1977), and although chromatin distribution within the nucleus can account for the observed staining patterns, the significance of the chromatin distribution has never been explained. Since as many as eight densely stained nuclei were observed within zoosporangia seen in thin-section, it is likely that there are at least sixteen and perhaps thirty-two

zoospores per zoosporangium. The zoosporangia of C. virescens produce eight to thirty-two zoospores (e.g. Chapman, 1976), and it seems reasonable to suggest that the range in P. epiphyton is the same. No specific number has been reported in the literature.

The position of the zoosporangial escape pore and the material occluding this pore are important in comparing Phycopeltis with other chroolepidaceous algae. Although the pore can occur at either a terminal, subterminal or lateral position on the zoosporangium, the observation that the pore is never positioned sublaterally (i.e. adjacent to the ZPS) provides a point of comparison with C. virescens in which such a sublateral pore position occurs (e.g. Good and Chapman, 1978b). Also, the zoosporangia of C. virescens are smooth-walled rather than papillate as in P. epiphyton (Chapman, 1976). The material that occludes the escape pore of P. epiphyton and extends into the upper region of the zoosporangium as well, has also been observed in the pore of Trentepohlia (personal correspondence, Dr. L. E. Graham, University of Wisconsin) and Cephaleuros (Dr. R. L. Chapman, unpublished results). On the basis of the ultrastructural appearance of the pore material, it is likely that it contains protein and polysaccharide. Consideration of zoospore release indicates that the occluding material must be dispersed, probably through enzymatic degradation triggered by environmental conditions (viz. moisture).

The observations strongly suggest that the bulging of septal regions of both the zoosporangium and the pedicel initiated lateral wall tearing in P. epiphyton, and is comparable to the bulging of septal regions reported for C. virescens (Chapman, 1976). Further,

these bulging septal regions have also been described and illustrated by line drawings of Trentepohlia (e.g. Fritsch, 1965) and would be expected to occur in Stomatochroon as well. The opening of the outer septal ring (Fig. 46) presumably extends into the ZPS and continues until the inner area of the ZPS is reached. Thus, the central inner area of both pedicel and zoosporangium are the final point of attachment between the pedicel and zoosporangium, a point of attachment which remains secure until the last stage of abscission is triggered. That last stage must be the emergence of a central protuberance at the inner ring of the ZPS in the zoosporangium, and in the pedicel. The zoosporangium is literally pushed off the pedicel, and moisture is surely a factor in this last stage of zoosporangial abscission. In addition to the observable morphological changes, chemical (or more specifically enzymatic) changes also may be involved in the abscission process.

Zoospores

The observations on quadriflagellate zoospores of P. epiphyton are consistent with Thompson's (1959) investigation on Cephaleuros and Stomatochroon. The earlier light microscopic reports on Phycopeltis producing biflagellate zoospores (e.g. Printz, 1939) certainly resulted from the flagella of zoospores being tightly appressed and therefore not discernable with the light microscope. Although on several occasions quadriflagellate motile cells of P. epiphyton were observed with the light microscope, the actual process of flagellar separation was never seen. This could indicate that the process occurs quickly.

Besides flagellar number, the flagellar apparatus (i.e. the basal bodies and associated structures) is also of prime importance in comparing P. epiphyton with other chroolepidaceous algae and in phylogenetically placing the Chroolepidaceae within the green algae. The occurrence of flagellar caps at the proximal end of basal bodies has also been reported in the quadriflagellate zoospores of Cephaleuros (Chapman, 1977) and in the biflagellate motile cells of Trentepohlia (Graham and McBride, 1975). The distal band observed above basal bodies has been reported to occur in the motile cells of other green algae including Pediastrum (e.g. Hawkins and Leedale, 1971), Hydrodictyon (e.g. Marchant and Pickett-Heaps, 1972), Microthamnion (Watson, 1975), and Bryopsis (Hori, 1977); however, the attachment of this distal band to basal bodies reported in other algae has not been observed in P. epiphyton. Further, the distal band does not seem to be present in the zoospores of Cephaleuros (Dr. R. L. Chapman, personal communication).

Multilayered structures present in P. epiphyton zoospores are similar to those present in Trentepohlia (Graham and McBride, 1975) and Cephaleuros (Chapman, 1977). The term multilayered structure basically refers to a lamellar layered structure found at flagellar bases. The upper layer of a multilayered structure is composed of microtubules which extend posteriorly in the motile cell forming a spline (Carothers and Kreitner, 1968). A multilayered structure has been reported in the motile reproductive cells of liverworts (e.g. Carothers and Kreitner, 1967; Carothers, 1973, 1975; Kreitner and Carothers, 1976), hornworts (e.g. Moser and Kreitner, 1970;

Carothers et al., 1977; Moser et al., 1977), mosses (e.g. Paolillo, 1965; Paolillo et al., 1968a, b; Lai and Bell, 1975), pteridophytes (e.g. Duckett and Bell, 1969; Bell et al., 1971; Duckett, 1973, 1975; Robbins and Carothers, 1978), cycads (e.g. Norstog, 1974, 1975) and in the motile reproductive cells of certain green algae including Coleochaete (McBride, 1971; Pickett-Heaps and Marchant, 1972), Chaetosphaeridium (Moestrup, 1974), Trentepohlia (Graham and McBride, 1975), Klebsormidium (e.g. Pickett-Heaps, 1975a), and Cephaleuros (Chapman, 1977). In addition, although the motile cells of Chara and Nitella contain a set of spline microtubules (microtubular band) which end at the flagellar bases, the lamellar layers of a multilayered structure are absent (Pickett-Heaps, 1968; Turner, 1968; Moestrup, 1970). According to Moestrup (1974), although the spline in Chara lacks lamellar layers, it is ontogenetically comparable to a multilayered structure. Graham and McBride (1975) suggested (based on electron micrographs in Turner's work, 1968) that the multilayered structure in Nitella is either rudimentary or vestigial.

The zoospores of P. epiphyton and other chroolepidaceous algae are unique in several ways. First, whereas the motile reproductive cells of archegoniate plants and certain green algae contain one multilayered structure, the quadriflagellate zoospores as well as biflagellate gametes of P. epiphyton and C. virescens (Chapman, 1977), contain at least two multilayered structures. Further, the available evidence indicates that the number of multilayered structures is not directly related to the number of flagella possessed by a motile cell.

All the green algae (other than the chroolepidaceous algae), as well as bryophytes and the lycopods Lycopodium and Selaginella, produce biflagellate motile reproductive cells that contain one multilayered structure. However, the motile cell flagella number of other vascular archegoniate plants ranges from approximately 15 to 20 in the lycopod Isoetes (Thomas and Duckett, 1976) to approximately 10,000 to 12,000 in the cycad Zamia (Norstog, 1975) and these multiflagellate motile cells also contain just one multilayered structure.

Second, the position of the two multilayered structures in quadriflagellate cells of the Chroolepidaceae is different from the position of the multilayered structure found in other reproductive motile plant cells. In the zoospores of both C. virescens and P. epiphyton each multilayered structure abuts at an acute angle on a portion of the proximal side of a basal body, whereas in other motile reproductive cells the multilayered structure is positioned beneath basal bodies.

Third, the tightly appressed paired flagella of zoospores, as well as the separation of these paired flagella, has only been reported to occur in the Chroolepidaceae.

Fourth, as expected, the flagella have the almost universal "9+2" microtubule arrangement found in other eukaryotes, but also bear unusual lateral flagellar keels. The presence of these keels in the motile cells of all chroolepidaceous algae studies thus far reinforces the suggestion (Graham and McBride, 1975; Chapman and Good, 1977) that their presence is a useful character for separating the Chroolepidaceae from other algal families. Since the keels extend throughout most of

the length of flagella, they could increase the swimming efficiency of the cell, as suggested by Graham and McBride (1975). The microtubule-like structures, or "microtubule-like fibers" in the terminology of Graham and McBride (1975), in these keels are difficult to interpret because they have not been consistently observed and could be an artifact resulting from specimen preparation. However, if the microtubule-like structures are not artifacts, then they could function in supporting the flagellar keels (Graham and McBride, 1975).

The morphology of multilayered structures in Phycopeltis is quite comparable to the morphology of those observed in other green algae, although not all multilayered structures are the same. In fact, the number of lamellar layers in a multilayered structure is variable. Ever since Heitz (1959) used the term Dreiergruppe to describe a new three-layered cytoplasmic organelle present beneath the flagellar bases of liverwort motile cells, and Carothers and Kreitner (1967) used the term Vierergruppe to describe a four-layered structure present beneath the flagellar bases of liverwort motile cells, the number of lamellar layers in a multilayered structure has been controversial. Paolillo et al. (1968a) felt that the term multilayered structure was preferable to both Dreiergruppe and Vierergruppe. Subsequent investigations on a variety of different plants strongly suggest that, depending on the developmental stage of a motile cell, some or even all of the strata of a multilayered structure can be transitory (e.g. Kreitner, 1977; Robbins and Carothers, 1978). Further, there is at least one report (Kreitner and Carothers, 1976) of the disorganization of a multilayered structure being reversible

(i.e. loss and subsequent gain of malellar layers). It would be interesting to observe if the multilayered structures persist in mature (i.e. released) zoospores of Phycopeltis and in the zoospores of Cephaleuros as well.

It has been suggested that spline microtubules in the motile cells of algae and archegoniate plants form a cytoskeleton that functions in maintaining the overall shape of the cell, especially during passage through a constricted pore (e.g. Duckett, 1975). Presumably, the splines in the zoospores of Phycopeltis and Cephaleuros have a similar function.

The symmetry (or lack of symmetry) of a zoospore is directly related to the structures comprising the flagellar apparatus and is considered by many to have phylogenetic implications. As defined here, an entity is symmetrical if, by central division, the resulting halves contain similar structures that are positioned at equivalent locations with the same orientation. An entity is asymmetrical if the resulting halves do not contain similar structures or the halves do contain similar structures but they are not positioned at equivalent locations with the same orientation. Employing these definitions, the zoospores of Phycopeltis are asymmetrical, as are those of Cephaleuros.

Gametangia and Gametes

Overall, the production of gametangia and gametes was in several ways similar to the production of zoosporangia and zoospores. The apparent absence of a large lightly stained nucleus like that observed

in zoosporangia prior to karyokinesis may have been the result of my not observing this developmental stage. Since observations on zoosporangia were far more numerous than those on gametangia, this explanation seems likely. Also, formation and disappearance of exit pore material in gametangia are comparable to those seen in zoosporangia. The gametes contained cellular organelles and cytoplasmic inclusions similar to those in zoospores and the absence of an eye-spot again confirmed earlier light microscopic reports on the absence of eye-spots in the motile cells of Phycopeltis (e.g. Printz, 1939).

The observations indicate that the primary morphological difference between the zoospores and gametes of P. epiphyton is that the gametes are biflagellate. This observation is wholly consistent with the report by Thompson (1959) that the gametes of both Cephaleuros and Stomatochroon are biflagellate. However, the report by Graham and McBride (1975) on the motile cells of Trentepohlia is not consistent with other chroolepidaceous algae. These authors worked with biflagellate motile cells that they believed to be zoospores. In view of Thompson's 1959 work and the present data on Phycopeltis, it seems highly probably that these biflagellate cells were gametes.

Since in P. epiphyton the distal band (previously discussed) found above the two upper basal bodies in zoospores is also present above the two basal bodies in gametes, it is interesting to note that this distal band is apparently absent from the biflagellate cells of Trentepohlia (Graham and McBride, 1975) and in the biflagellate cells (gametes) of Cephaleuros (Dr. R. L. Chapman, unpublished results).

The significance of the apparent absence of the distal band in certain chroolepidaceous algae remains unknown and future studies on the quadriflagellate cells of Trentepohlia as well as the gametes and zoospores of Stomatochroon are certainly needed.

The presence of two multilayered structures in gametes of P. epiphyton is consistent with the reports of two multilayered structures occurring in the biflagellate cells of Trentepohlia (Graham and McBride, 1975) and Cephaleuros (Dr. R. L. Chapman, unpublished results). One would expect that two multilayered structures are present in the motile cells of Stomatochroon as well.

The fine structure of reproductive motile cells is one character that has proven useful for studies of evolutionary trends in green algae (e.g. Manton, 1965; Moestrup, 1974, 1975; Pickett-Heaps, 1975b; Stewart and Mattox, 1975). Those green algae that have been suggested to be on a direct line to archegoniate plants produce motile reproductive cells (if motile cells are produced) that are asymmetrical, contain one multilayered structure (with or without the lamellar layered portion), and possess flagella that are laterally inserted, whereas those green algae not on this proposed evolutionary line produce motile reproductive cells (if motile cells are produced) that are symmetrical, do not contain a multilayered structure, and possess anteriorly inserted flagella. The motile cells of the Chroolepidaceae are unique among the green algae because not only do they contain two multilayered structures but also have anteriorly inserted flagella. In addition to being unique among the green algae, Stewart and Mattox (1975) also believed, based on the report by Graham and McBride (1974),

that the structure of the motile cells of Trentepohlia "is less like the flagellated cells of archegoniate land plants than are those of the Coleochaetales and Charales." The observations on the motile cells of Phycopeltis and on Cephaleuros (Chapman, 1977, and unpublished results) are certainly consistent with the opinion of Stewart and Mattox (1975); however, where should the chroolepidaceous algae be placed phylogenetically within the green algae? The excellent report by Stewart and Mattox (1975) failed to place the Chroolepidaceae in either the proposed "Charophyceae", on the evolutionary line to archegoniate plants or the "Chlorophyceae" not on the evolutionary line to archegoniate plants. Based solely on the fine structure of motile cells, one could suggest that the Chroolepidaceae are not on a direct evolutionary line to archegoniate plants, but rather split from that line after evolution of the multilayered structures. The available fossil evidence indicates that the Charophyceae had a much earlier geologic history (e.g. Silurian, Devonian, Jurassic) than the chroolepidaceous algae which first appeared in the fossil record during the Tertiary (e.g. Kirchheimer, 1942; Bold and Wynne, 1978). Stewart and Mattox (1975) suggested that it was conceivable that some advanced algal forms would not fit into either of their proposed classes. The observations on P. epiphyton together with those on Cephaleuros and Trentepohlia indicate that the Chroolepidaceae cannot be accommodated in either the Chlorophyceae or Charophyceae sensu Stewart and Mattox (1975). The presence of multilayered structures in the Chroolepidaceae justifies the inclusion of this family in the Charophyceae which

should be amended accordingly. Thus, based on all the information available, it seems likely that the Chroolepidaceae are a recently evolved, highly specialized family which diverged from the Charophyceae line of green algal evolution and hence are not on the direct evolutionary line to archegoniate plants.

Concluding Statement

Although it is unnecessary to repeat the discussions and conclusions already presented, it is perhaps appropriate to reiterate a statement presented in the general introduction. Virtually unknown to most phycologists, Phycopeltis is an intriguing alga in an unusual family of green algae and although this work has revealed heretofore unknown information about Phycopeltis, several topics warranting future studies remain. For example, the elucidation of the life cycle, analysis of host specificity and the details of karyokinesis and cytokinesis are all suitable candidates for future investigation. Many of these questions are under continuing investigation and it is probable that additional answers will be forthcoming. However, as in any field of scientific endeavor, new information will undoubtedly generate new questions specific to Phycopeltis and other chroolepidaceous algae.

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Vita

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EXAMINATION AND THESIS REPORT

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Major Field: Botany

Title of Thesis: Ultrastructural and Biochemical Studies on the Epiphytic Subaerial Green Alga Phycopeltis epiphyton Millardet.

Approved:

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July 14, 1978